ENDOWMENT OF THE JOURNAL BY SIR JOSEPH VERCO

The Board of Editors have much pleasure in announcing the munificent endowment of the Australian Journal of Experimental Biology and Medical Science, with the sum of £5,000, by Sir Joseph Verco. This sum will be held in trust by the Council of the University of Adelaide, and the income derived therefrom will be devoted to sustaining the Journal, which will become the property of the University.

The Editors wish to express their appreciation of the assistance which has thus been rendered by Sir Joseph Verco to the furtherance of research in the sciences fundamental to Medicine in Australia. The purpose and scope of the Journal will be the same as heretofore, and it will be conducted by the same editorial committee. As hitherto, the Journal will be available for the publication of any research of merit in its particular field which may be submitted to it.

The Editors furthermore desire to record their sense of the indebtedness of Medical Science and Education generally to the services and generosity of Sir Joseph Verco. Since the foundation of the Medical School of the University of Adelaide Sir Joseph Verco has been actively associated with it, and we are very largely indebted to him for the progress which it has achieved.

He was born at Fullarton, in South Australia, seventy-five years ago, and received his early education at the Adelaide Educational Institution, conducted by the late Mr. John L. Young, and afterwards at St. Peter's College, winning the Young Exhibition. He received his medical education at St. Bartholomew's Hospital School, and graduated a Bachelor of Medicine of the University of London in 1875.

His student career was marked with the greatest distinction. He acted as House Physician at St. Bartholomew's Hospital during 1876. He became a Doctor of Medicine in the same year, and a Fellow of the Royal College of Surgeons, England, in 1877. He then returned to Adelaide, where he was was engaged in an extensive and distinguished practice for many years. His learning and skill were at the disposal of the Medical School and the Adelaide Hospital. He was lecturer in Medicine at the University for twenty-seven years, and has acted as Honorary Physician and Consulting Physician at the Adelaide Hospital for forty-four years, and at the Adelaide Children's Hospital for forty-eight years. Throughout his busy life he has always shown the utmost interest

in the welfare of scientific societies in South Australia. He was for nineteen years President of the Royal Society of South Australia, and is at present Honorary Curator of the Section of Mollusca of the South Australian Museum. He has filled all the positions of honour that could be bestowed upon him by his fellow-practitioners, as President of the South Australian Branch of the British Medical Association in 1886-1887, and for four years during the war, and was President of the first Intercolonal Medical Congress held in Adelaide in 1887.

Upon retiring from practice he gave most generously of his time to the Medical School as Dean of the Medical Faculty and member of the Council of the University for several years. He took a leading part in founding the School of Dentistry.

He resigned the Deanship of the Faculty of Medicine to undertake the direction of the newly-created Faculty of Dentistry, of which he is still Dean. As a member of the University Council and the Advisory Committee of the Adelaide Hospital, however, his services are still rendered generously available in the interests of the School of Medicine.

His contributions to medical literature include many communications to the medical publications of Australia, and he was associated with the late Sir Edward Charles Stirling in the authorship of the article on Hydatid Disease in Allbutt's "System of Medicine." He also contributed widely to the Proceedings of the Royal Society of South Australia.

His gift is a happy expression of his devotion alike to Medicine and to Science.

THE ACTION OF CARBONIC ACID ON HAEMOGLOBIN

by

WILLIAM ALEXANDER OSBORNE

(From the Physiological Laboratory, University of Melbourne).

(Submitted for publication 16th May, 1926.)

That haemoglobin is a salt compounded of a protein base, globin, and a complex organic acid, haematin, haemochromogen, or haem, is well known. The addition of acetic acid, as in the class exercise for making "acid haematin," produces globin acetate and liberates the complex haem, which like so many organic acids is soluble in ether, whilst the salts are insoluble. The object of the present investigation was to discover whether carbonic acid has a similar action, displacing the haem from its globin combination. The experiments were made with blood freshly drawn from the carotid artery of an anaesthetized dog.

If carbon dioxide gas, washed in aqueous sodium bicarbonate suspension, is bubbled through diluted blood the characteristic absorption band in the red of acid haematin will be detected on spectroscopic examination after several hours. That this is not methaemoglobin is shown by having a layer of ether above the blood, which may be undiluted, during the passage of the gas. This ether becomes eventually coloured, and gives the typical absorption band of haem in ethereal solution.

Three parts blood were added to 100 parts water supersaturated with CO_2. The mixture was shaken to get ride of excess CO_2, and sealed in a glass vessel suitable for spectroscopic examination. It was found that the absorption band in the red was not visible until five hours had elapsed. That this delay is due to the haemoglobin, and not to the qualities of the carbonic acid, is shown by adding blood to excess of an organic acid of approximately the same P_H. I have been unable to find in physico-chemical literature any figure for the P_H of water saturated at different temperatures with CO_2. The dissociation constant has been determined by more than one observer, but the results do not correspond. Taking the constant found by Kendall (1) for 18°C., namely, $3{\cdot}12 \times 10{\cdot}7$, and the known solubility in water at that temperature, the P_H should be 3·9. Dr. J. M. Lewis in my laboratory has found by the electric method, using a Leeds Northrup potentiometer and minimal contact wire electrodes, values for the P_H that lie

close to 5. Blood was added to excess of acetic acid of P_{H_1}, and it was found that the slow change of colour and appearance of the spectrum of acid haematin were quite comparable with the action of carbon dioxide.

Dilute blood (3 in 100) was placed in a small rectangular bottle, and this was inserted in a Leonard Hill apparatus for studying the action of high gas pressures on small animals, in such a way that the spectrum could easily be seen through the thick glass windows. After subjection to 15 atmospheres CO_2 for twenty minutes no change was observable in colour or spectrum; longer exposures were unfortunately rendered impossible by a defect in the apparatus.

Blood diluted with water containing CO_2 at various tensions down to 10% atmosphere similarly showed the presence of haematin if sufficient time was allowed.

There is, however, a method by which the immediate formation of haematin can be detected, and that is to view highly diluted blood through a long tube. The dilution I have chosen is 1 in 3600, and the tube is 52 inches in length. The ends are closed with flat glasses from a polarimeter tube, and contact with metal is avoided on account of the ease of methaemoglobin formation. If the blood be freshly drawn from the carotid, and diluted with distilled water recently boiled, no band is seen in the red. But if the water contain a trace of CO_2 the band appears. Stale blood, twenty-four hours old and more, diluted with boiled water, gives a simlar band in the red, though the possibility of methaemoglobin formation must not be forgotten.

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ACUTE ENCEPHALITIS (X-DISEASE) AT BROKEN HILL

PROBABLE SUCCESSFUL TRANSMISSION TO SHEEP

by

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(From the General Hospital, Broken Hill, and the Department of Pathology, University of Adelaide).

(Submitted for publication 13th August, 1926.)

In January and February, 1925, cases diagnosed as acute encephalitis (X-disease) again appeared at Broken Hill. The symptoms and signs manifested corresponded with those already described in previous reports (1 to 6). The following points may again be emphasized: The considerable mortality, the high temperature often reached, the presence of coma, and often convulsions, the relative absence of eye lesions, the fairly rapid progress to death of the graver cases, and a general resemblance to cerebro-spinal fever, except that the cerebro-spinal fluid is clear instead of turbid. Increases in the leucocyte counts were also noted. We are of opinion that this symptomatology does not correspond with that of encephalitis lethargica. At the time these cases occurred there was no increase in ordinary cases of anterior poliomyelitis in the district, which increase would have been expected had these cases been encephalitic examples of this disease. An enquiry by one of us showed that only one case of undoubted ordinary infantile paralysis was reported in the district between November 1, 1924, and May 31, 1925. The apparent conveyance of the virus of this disease to sheep is also an important point of difference. We find in these further cases, and in the inoculation results evidence confirmatory of the view that the disease is a definite entity.

When several of these cases of encephalitis had appeared, one of us (J.K.) communicated with the other, and as a result it was determined to try to convey the virus of the disease by intracerebral inoculations to sheep. It will be remembered that in the first outbreaks of the disease in 1917 and 1918 the virus was apparently successfully conveyed to a series of sheep after its establishment in the monkey (1, 2, 6). We determined in this epidemic, if possible, to convey the

disease directly from the human victim to sheep. Though the stress of work at Broken Hill was heavy at the time, inoculations were made into two sheep, as detailed later on, with what appeared to be successful results. A second series of inoculations were made from one of the first sheep, and a successful "take" in the third generation was also obtained, but not in the fourth. At this stage the press of other work, the difficulties entailed in obtaining animals and looking after them, and the expense, prevented a continuance of the experimental investigations. An effort was made to transplant the virus to Adelaide, but, unfortunately, the inoculations there were completely without effect.

It is realized that much more work might have been done in connection with the sheep successfuly inoculated; thus an examination of the brains of the animals that succumbed might have been much more extensive, with the object of showing still more clearly that no secondary bacterial organism was playing a part in the process. It was impossible, however, under the circumstances, to do much more than make the actual intracerebral inoculations, and after death ordinary blood-serum inoculations from the brains. It is realized that the failure to carry out all the checks possible detracts from the importance of the results. Nevertheless, we feel that what has been done should be put on record, and we hope that others who may have a chance to repeat these experiments under better conditions will avail themselves of any opportunity that arises.

Details of Inoculation Experiments.

Portions of the cerebrum, cerebellum, mid-brain, pons, medulla, and cervical cord of Lucy S. (Case 3), removed aseptically, were emulsified in a sterile mortar with 50% glycerine and water. The resultant product was used the same day to inoculate Sheep 1 and 2. In all cases the procedure used was the same, except that an anaesthetic was only used for Sheep 1 and 2. The hair was removed, the scalp thoroughly cleansed, and through a small incision a perforation was made through the skull in the parietal region (the perforation was made with a drill). It was found quite easy to tell when the skull was almost perforated, and a needle fixed to a 2 cc. syringe was used to inject 1½ cc. of the emulsion deeply and steadily into the brain substance. In most cases there was a little temporary unsteadiness or rigidity on one side as a result of this inoculation, although this cleared up rapidly, except in Sheep 1, in which it persisted for some days.

Sheep 1, inoculated on March 3, 1925, from the above emulsion, did not stand up for three days after recovery from the anaesthetic, and was very unsteady. It walked better the fifth day, and took food well for 24 hours, but then went off food again, became unsteady, and *died with general convulsions on March 11, 1925.*

Sheep 2 was inoculated on March 3, 1925. There was no noticeable change

until the day before death, when a disinclination to feed was noticed. On the day of death, March 12, 1925, it kept wandering around in a circle, with the head retracted, and finally died after several general convulsions. (Both these animals were about a year old.)

Sheep 3 was inoculated with the emulsified brain of Sheep 2 on March 15, 1925, and died with similar symptoms to Sheep 2 on March 21, 1925.

Sheep 4 was inoculated with the emulsified brain of Sheep 3 on March 22, 1925. It kept apparently well for eight days, and died after convulsive attacks for 24 hours on March 31, 1925.

Sheep 5 was re-inoculated from the brain of Sheep 4 on April 1, 1925, but suffered no ill-effects. (It had been inoculated with the brain of Sheep 3 on March 15, 1925, without ill-effect.)

Sheep 3, 4, and 5 were lambs about three months old.

The brains from Sheep 1, 2, 3, and 4 were removed with strict aseptic precautions immediately after death, and preserved in 50% glycerine and water. They all showed the most intense pial congestion, with not the least evidence of suppuration in the needle track or elsewhere.

Complete bacteriological examinations were not carried out, but inoculations on to blood-serum from the needle track and surrounding brain tissue were made in each case with no growth resulting.

Microscopic Examination of the Nervous Tissue of the Sheep.

Sheep 1. Sections were made of the spinal cord, the medulla, pons, floor of the fourth ventricle, frontal and parietal regions. In all these perivenous cellular sheaths were present, sometimes to a marked degree. The cells present were lymphocytes, large mononucleated cells, and polymorpho-nuclears. No definite neuronophagocytosis was detected. No infiltration of the pia mater was present in the spinal cord, but there was some increase of the lymphocytes and a few polymorpho-nuclears in the meshes of the pia mater of the parietal region, and in some of the sulci perivenous sheaths were present. There were some cellular islands in the pons.

Sheep 2 showed similar lesions in sections of similar portions of the spinal cord and brain. There was marked sheathing of the vessels in places, such as in the medulla. Polymorpho-nuclears were fairly evident amongst the lymphocytes, etc. There were some islands of cells in the pons.

Sheep 3 yielded similar results. In the pia mater of the parietal region there was considerable infiltration with small cells, chiefly lymphocytes and large mononuclears.

In *Sheep 4* there had been a haemorrhage in the frontal region from the trauma of the injection. There remained some old blood-clot, surrounding which was an angiomatous-looking inflammatory reaction, consisting of large, irregular,

thin-walled vessels, between which were swollen glial cells, some connective tissue cells, etc. Beyond the injured and reactionary area lymphoid perivenous sheaths were obvious. In the temporal region there were a few well-marked sheaths, but these were almost absent in the parietal area. No lesions were detected in the basal ganglia, cerebellum, or spinal cord, but in the pons were slight indications of perivenous sheaths.

DEATHS.

Case 1. Gladys K., aged 3 years, was admitted on January 17, 1925, in an unconscious condition, with a history of vomiting and drowsiness for some days; convulsions and delirium occurred 3 days previous to admission. The heart, lungs, and abdomen showed nothing abnormal. Head retraction and dyspnoea were present. The temperature was 103·2°F., the pulse 144, and respirations 44. Lumbar puncture yielded clear fluid under pressure. No organisms were detected in it by smears or culture. The child died without rallying.

Case 2. Mary P., aged 2 years and 6 months, was admitted on February 2, 1925, with a history of vomiting for two days and with convulsions on the day of admission. The bowels were quite regular. She had been well previously. On examination nothing abnormal was detected in the chest or abdomen. Head retraction was present, and Kernig's sign. Lumbar puncture yielded a clear fluid, with the pressure not much increased, and containing no organisms. The temperature was 104°F., the pulse 160, and respirations 34. Next day she seemed slightly easier. On February 4 she had a severe convulsion in the morning, and died, the temperature reaching 106·6°F. at the end. At the post-mortem examination the pial vessels were found to be very congested. There was no evidence of tuberculosis or suppurative meningitis. The mesenteric glands were enlarged. No other lesions were detected. Microscopical sections from four different parts of the brain showed the presence of definite islands of small cells, but perivenous sheaths were not detected. The small cells were sometimes shrunken, and occasional polymorphonuclears were amongst them. In one section, a small degenerated area, probably an infarct, was seen with shrunken nuclei and disappearance of the cerebral tissue.

Case 3. (From this case the sheep inoculations were made.) Lucy S., aged 2 years and 10 months, was admitted on March 1, 1925. She had had whooping-cough ten months before, but since then had been quite well up to two days ago. For the last two days she has had "turns" each day, lasting up to six minutes at a time, and three convulsions, followed by general twitchings during the day before admission. The bowels had been opened once daily. She had refused food since she became ill. Examination showed that she was in a semi-comatose condition, with generalized twitchings. Head retraction and Kernig's sign were present. Nothing abnormal was detected in the chest or abdomen. The urine

showed no abnormality. The temperature was 101·6°F., the pulse 140, and respirations 48. Next day her condition was worse, although the twitchings were not so severe. There was left abducent paralysis. Lumbar puncture yielded 60 cc. of clear fluid under slightly increased pressure. This fluid contained no increase of globulin, no pus cells, and no organisms either in smears or on culture. The temperature, 101·6°F. on admission, rose in 12 hours to 105°F., and maintained a high level, rising to 106° a few hours before death, which occurred 36 hours after admission. At the post-mortem examination, 12 hours after death, the brain was removed with strict aseptic precautions. Very marked congestion of the vessels of the pia mater was present, both at the vertex and the base. There was no evidence of tuberculous or suppurative meningitis, and the other organs were healthy. Microscopic examination of sections from the brain and spinal cord failed to reveal the presence of perivenous sheaths of cells or of cellular islands. These sections included the basal nuclei. There was marked capillary congestion in the frontal and occipital areas.

Case 4. Guiseppe G., aged 26 years, was admitted on March 28, 1925. He was a miner, living in Broken Hill. There was a history of headache and loss of appetite five days before, but he had worked up to two days before admission. On the day before admission he was drowsy, and complained of headache. He became worse, hot, and delirious, and had vomiting and abdominal pain. Examination showed a well-built foreigner, who was very drowsy, and did not answer questions properly. The temperature was 105°F., the pulse 104, and respirations 28. The legs were held rigid, so that the knee-jerks could not be elicited. On March 30 he was still running a temperature up to 106°F. He could not be roused, but picked at the bed-clothes, and waved his right arm round. The leucocytes numbered 25,000 per c.mm. The Widal reaction was negative. By March 31 his condition had become gradually worse, but the pulse remained good up to the last, when death occurred from respiratory arrest. At the post-mortem examination the brain was very congested. The vessels of the pia mater were intensely engorged. Nothing abnormal of note was detected in the other organs, including the intestine. Microscopic sections of the frontal and occipital regions showed only congestion of the pial vessels. In the temporal region round one vein were a small number of lymphocytes, and near the surface a small island of degenerating small cells with pycnotic nuclei, some of the cells possibly polymorphonuclears. In the parietal area near a vein at the periphery there was a very small cellular island with irregular nuclei. No lesions were detected in the basal ganglia. In the pons there were a number of diffuse cellular islands scattered amongst the nerve cells, their nuclei irregular or flattened; in several places the brain tissue seemed fragmented or fibrillar, and scattered through it were irregular nucleated cells, some of them lymphocytes. In the medulla there were some scattered diffuse cellular islands with irregular nuclei.

Case 5. Allan R., aged 25 years, was admitted on April 26, 1925. He was

a miner, living in Broken Hill. He had been quite well until four days before, when on returning from work he complained of severe pain over the right eye and slight abdominal pain. He vomited the same evening. He stayed in bed next day, with headache, abdominal pain, vomiting, and constipation. He became hot and feverish the same night, and could not sleep. The day before admission he had had a rigour, and became drowsy. On the morning of admission he became strange, stared about him, and would not speak. On examination he was drowsy, and did not answer questions. His temperature was 102°F., the pulse 132, and respirations 26. Nothing abnormal was detected in the heart, lungs, and abdomen. The white blood count was 13,750 per c.mm. On April 27 he could not be roused. There was plucking at the bed-clothes. In the afternoon the temperature rose to 106·2°F., the pulse to 152, and respirations to 32. On April 28 the temperature went up to 106·2°F. again. His general condition was worse. There was frequent spasmodic stiffening of the body, limbs, and neck. He gradually became more and more deeply unconscious, and died the same day. A blood-culture was negative. At the post-mortem examination all the vessels of the brain were intensely congested, but there was no exudate or other evidence of meningitis. The other organs were normal, including the intestine. Sections showed congestion of the vessels in the parietal and occipital regions, and in the medulla small cellular island and a tendency occasionally to an increase of cells in the perivenous spaces.

Case 6, Tibooburra Case (by kind permission of Dr. Keyes). J.S., a girl aged 8 years, was admitted at 3 p.m. on January 30, 1925, in a comatose condition, with a temperation of 102·4°F., pulse 112, and respirations 24. The mother stated that the child complained of not feeling well two days previously. As she was subject to "bilious attacks" she took little notice, and treated her for such (oil and restricted diet). The child vomited several times. She got out of her cot on the second night, which appears to have been the first symptom of cerebral involvement. Drowsiness came on then, and on the morning of admission the mother was frightened, which caused her to bring the child to the hospital. On examination the child was comatose, and definitely gave Kernig's sign. Heart and lung signs were absent; the urine was clear. The Babinski response was doubtful, and there is no record of subsequent Babinskis being positive. She was put on a mixture of hexamine 5 gr. 4-hourly. During the night the child was delirious and talkative. On the second day the temperature came down to normal, pulse 120, respirations 28. At 9 a.m. she had a convulsive attack. The left arm was stiff, the hand clenched, and jaws fixed. There was no opisthotonos. The attack lasted a few minutes. At 10 a.m. lumbar puncture was performed, which gave fluid under considerable pressure, but clear. From this time on spasms became more frequent, until by evening they came on every ten minutes. Opisthotonos increased progressively. At 9 p.m. the temperature was 106°F. The child was put into a cold pack, which reduced the temperature to 104·2°F.

Lumbar puncture was performed again in the evening, and the fluid obtained was under slight pressure. From then on the spasms were very severe until midnight, when they became less frequent and slightly less severe. There was extreme exhaustion. A 9 a.m. next morning the temperature was 104·2°F., pulse 140, respirations 36. Her condition seemed slightly better. The spasms ceased until midday, but during the afternoon she had about four spasms. Strophanthin 1/500 gr. was given at 11.45 p.m., and again at 3.30 a.m. Digitalis was administered at 1.45 a.m. by mouth. During this day she took slight nourishment, being able to swallow fairly well. During the night she had no spasm, but the pulse was almost uncountable. The temperature at 11 p.m. was 101·4° In the morning she lay in a stuporose condition with a temperature of 104·5°F. Strophanthin was administered at 10 a.m. Her condition became more and more stuporose, and she died at 1 p.m.

RECOVERIES.

Case 7. W.H.R., a male, aged 17, was admitted on February 22, 1925, in an unconscious state, from a station 240 miles away on the Queensland border. Four days previously he complained of pain in the back of the neck and between the shoulders, and was thought to be suffering from sunstroke. The day before admission he became unconscious, and was restrained with difficulty. He had had an attack of influenza two weeks before. Examination showed a thin-faced boy in an unconscious state. He would lie still for five to ten minutes, breathing through the mouth, and then stiffen the body and legs. There was twitching of the left side of the face. Temperature 102°F., pulse 96 (varying considerably), respirations 28 and of the Cheyne-Stokes type when quiet. He kept his eyes shut, and resented having them opened. The pupils were small, equal, and reacted to light. There was nothing abnormal detected in the chest and abdomen. The urine contained a faint cloud of albumen, but no pus or organisms. By lumbar puncture 10 cc. of clear fluid were withdrawn under considerable pressure, which contained an increased number of cells (lmphocytes predominating), but no increase in globulin. There were no organisms present. The white blood cells numbered 17,500 per c.mm. The Wassermann test was negative. On February 23 he was still quite unconscious. On Febrary 25 he was conscious, but drowsy and listless, with the temperature normal. On March 1 he was much improved, with no headache, and talked rationally. On March 8 he was up, but his mentality seemed below normal.

Case 8. George G., aged 4½ years, was admitted on March 2, 1925. He had been quite well till the previous day, when he had headache and abdominal pain, followed by convulsions and vomiting. The bowels were normal, and there had been no dietetic errors. Examination showed a well-nourished boy with generalized twitchings. Head retraction and Kernig's sign were present. The heart,

lungs, and abdomen showed nothing abnormal. By lumbar puncture clear fluid was withdrawn under slightly increased pressure, which showed an increased cellular content but no organisms. The temperature was 103·6°F., pulse 140, respiration 40.

Progress: The convulsions and rise of temperature only lasted for two days after admission. He then gradually improved, and was discharged cured on March 30, 1925. He vomited frequently during the acute stage of the illness, and had a screaming attack during convalescence. He was kept under the influence of sedatives, which seemed to have a marked influence on the convulsive phenomena.

Case 9. Alfred G. A., aged 4 months, was admitted on March 3, 1925. He was a perfectly healthy breast-fed baby until the previous day, when he seemed feverish and had twitching attacks. The bowels were quite normal. Examination showed a healthy-looking child with twitching of the face and hands. Kernig's sign and head retraction were present. He had a convulsion lasting three minutes shortly after admission. Lumbar puncture yielded clear fluid under slight increase of pressure. Nothing abnormal was found in the heart, lungs, and abdomen. Temperature 104·6°F., pulse 166, respirations 60.

Progress: There were twitchings with occasional convulsions until March 8, 1925, although the temperatures began to fall after two days. He was unable to swallow, and was fed with the stomach tube during this period. After March 9, 1925, he progressed well, and was discharged on March 29 apparently quite well.

Case 10. Jessie B., aged 2$\frac{11}{12}$ years, was admitted on March 11, 1925. She had been well up to five days before admission, and then vomited and had a twitching attack. She was drowsy until three days previously, when she became very drowsy, with twitching of the face and arms. Two days before admission she became unconscious, and on the morning of admission the head had become bent back and the back arched. Examination showed marked head retraction, dyspnoea, colour poor, and Kernig's sign present. No paralysis was found or abnormality in other organs. Temperature 104·4°F., pulse 160, respirations 48. Lumbar puncture gave a clear fluid under slight pressure, with the globulin increased, and there was also increase in the cellular contents. There were no organisms in smear or culture. The urine was normal.

Progress: On March 13 the patient was slightly better, but would not take drinks. There was slight neck rigidity, with face and hand twitching. She had to be fed by stomach tube for fourteen days. The general condition gradually improved, and the temperature returned to normal, but the child had a vacant look, and took no notice of surrounding objects. Although there was no actual paralysis there was spasticity of the muscles of the neck and arm, and on her discharge on May 25, 1925, she could not walk properly. Some months later the

child was able to walk and the mental condition had improved, but was still not quite normal.

SUMMARY.

1. Further cases of a form of acute encephalitis, occurring in the late summer in a dry, hot climate, are reported. The mortality was high.

2. The disease appears to have been conveyed to sheep by intracerebral inoculation from a human case, and transmitted to two other sheep in series.

3. Microscopic lesions in the human brains were slight, and in the case from which the sheep were inoculated only congestion has been detected.

4. As in previous reports, a sheep found refractory to a first inoculation has proved refractory to a second.

5. The results obtained support the view that this disease is a distinct entity, unless further and repeated attempts to convey the virus of ordinary acute polio-encephalitis to sheep eventually yield positive results.

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THE MILK OF AUSTRALIAN WOMEN

by

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with the assistance of

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(Received for publication 10th August, 1926.)

The work described in this paper has been carried out to determine the variability of the milk secreted by normal women of Australian birth and European stock after lactation has reached a steady state. The connection between properties of the milk and certain factors relating to the mother and the child have also been examined. The variability during the earlier stages of lactation has been dealt with in a previous paper (Wardlaw, 16).

The women from whom the samples were taken were all adequately suckling their infants. The infant in each case appeared to be normal, and no history of any serious illness was obtained for it. In a few instances the mother's milk had been supplemented on certain occasions by other food, but in no case was the supplement habitual, nor had it been made use of during the period immediately preceding the examination.

A history was obtained from each woman, which included statements of her age, the number of children she had borne and suckled, the age and sex of the youngest child, the number of times it was suckled per day, and the nature of its motions. Information was also collected with regard to the prenatal condition of the mother, the nature of her last confinement, her occupation, diet, and other matters which are not dealt with in the present paper.

The infants were weighed naked to the nearest half-ounce at the institutions at which the samples of milk were collected.

The samples were obtained during the period between March and November, 1923. One sample only was taken from each woman. Except in one or two cases, in which a breast pump was used, the milk was obtained by massage of the breast. Care was taken to empty the breasts as completely as possible, not only to measure as accurately as possible the volume of milk obtainable in each case, but also on account of the variation of the fat content with the degree of emptying of the gland. It has long been known that the percentage of fat in

the portion of the sample first withdrawn from the breast may be less than half that in the last portions (Söldner, 14). The similar behaviour of the concentration of fat in cow's milk during milking is also well known.

The concentrations of the other constituents of the milk are generally supposed not to be sensibly affected by the stage of emptying of the mammary gland. Talbot (15), however, has recorded a slight increase in the percentage of lactose as the breast is emptied.

Except in six cases, in which the samples were obtained from both breasts, the samples were obtained from one breast. The six samples obtained from two breasts are distinguished by asterisks in the table of results. The volumes in these cases have been halved to make them comparable with the figures for the other samples.

The subjects were instructed to feed their infants from one breast only at 6 a.m. on the day of collection of the sample, and to refrain from using either breast again until after the collection of the sample, which took place at about 10 a.m. Except where both breasts were emptied, the sample was obtained from the breast which had not been used last. The samples were therefore obtained after a minimum period of rest of four hours, although in most cases the period of rest must have been considerably greater than this.

The volumes of the samples obtained were measured to the nearest cubic centimeter. The analyses were commenced within about an hour of collection.

The specific gravity of the samples was determined by means of a pycnometer at a temperature of 25°C. The figures given are those compared with water at 25°C. The pycnometer used held about 2 cc. of milk.

The freezing point was determined with a Beckman thermometer by the method previously described by one of us (Wardlaw, 17).

The total solids were determined by weighing the solids obtained from the milk delivered from a calibrated 1 cc. pipette. The milk was dried for about six hours in a water oven.

Fat was determined on 5 cc. samples of milk from a calibrated pipette by the Röse-Gottlieb method.

Ash was determined on 5 cc. samples delivered from the same pipette as was used for the determinations of fat. The samples were dried in porcelain crucibles in a water oven, then ashed at a low red heat in a gas muffle. In cases where difficulty was experienced in getting rid of the last traces of carbon at the low temperature used, a little pure ammonium nitrate was added after cooling, and the heating repeated.

Lactose was determined by the Folin-McEllroy method (Folin and Denis, 5). One volume of milk diluted with nine volumes of water was used for the determination.

The percentages of protein were computed as follows: The difference between the percentage of total solids and the sum of the percentages of the individual

constituents gives the percentage of protein and "extractives." From this the number 0·73 was subtracted as the average percentage of extractives.

The figure for the average percentage of extractives was obtained by subtracting the sum of the average percentages of lactose and ash for the present results (6·64%) from the average percentages of lactose, ash, and extractives (7·37%), obtained in a previous investigation (Wardlaw, 16), in which the protein was estimated directly by the method of Sikes (13) during the second week of lactation. Camerer and Söldner (1) have shown that this latter sum remains practically constant from this stage of lactation onwards.

RESULTS.

The results of the various estimations are recorded in the following table. Certain particulars with regard to the mother and child are also shown. The results are arranged in order of the age of the child or stage of lactation of the mother. Where several specimens of milk have been obtained at the same stage of lactation, they have been arranged in order of the volumes of the samples.

The figures in the different columns are as follows: (1) Serial number of sample; (2) Age of child in weeks to nearest half-week; (3) Age of mother in years to nearest year; (4) Weight of child at time of sampling the nearest tenth of a kilogram; (5) The number of times per day the child was suckled; (6) The volume of the sample to the nearest cubic centimetre; (7) The specific gravity at 25°C. compared with water at the same temperature; (8) The depression of freezing point; (9) Percentage of total solid matter; (10) percentage of fat; (11) Percentage of solids-not-fat; (12) Percentage of lactose expressed as anhydride; (13) Percentage of ash; (14) percentage of protein.

The table shows the results of examination of 120 specimens of human milk. The figure for the depression of freezing point is not available for some of the samples.

TABLE 1.

Composition and Properties of Human Milk from First Month of Lactation.

Number of Samples.	Age of Child, Weeks.	Age of Mother, Years.	Weight of Child, Kilograms.	Volume of Sample, c.cm.	Specific Gravity, 25°/25°.	Dep. of Freezing Point.	Total Solids, per cent.	Fat, per cent.	Solids, not Fat, per cent.	Lactose anhyd., per cent.	Ash, per cent.	Protein, per cent.
25	[illegible]	32	2.63	46	1.0269	—	13.35	4.8	8.55	5.13	0.21	2.52
16	4	26	3.3	28	1.0287	—	13.35	5.32	8.03	6.50	0.17	0.65
87	4	21	3.4	34	1.0283	0.592	14.56	5.45	9.11	6.26	0.24	1.92
133	4	33	4.2	54	1.0312	0.579	13.23	4.29	8.94	6.74	0.13	1.20
99	4	20	4.2	100	1.0307	0.573	13.86	5.04	8.82	6.45	0.16	1.51
131	4	39	4.9	100	1.0332	0.577	11.70	2.59	9.11	6.60	0.21	1.60
120	4.5	24	3.5	72	1.0312	0.590	14.25	4.47	9.76	6.20	0.26	2.61
122	4.5	32	5.0	76	1.0287	0.593	14.94	5.95	8.99	6.60	0.20	1.50
117*	4.5	36	4.6	50	1.0325	0.604	12.31	3.15	9.16	6.65	0.19	1.62

Number of Samples.	Age of Child, Weeks.	Age of Mother, Years.	Weight of Child, Kilograms.	Volume of Sample, c.cm.	Specific Gravity, 25°/25°.	Dep. of Freezing Point.	Total Solids, per cent.	Fat, per cent.	Solids, not Fat, per cent.	Lactose anhyd., per cent.	Ash, per cent.	Protein, per cent.
27	5	29	3.7	40	1.0274	—	15.64	6.61	9.03	6.35	0.12	1.87
20	5	40	3.6	42	1.0301	—	12.94	4.24	8.70	6.76	0.14	1.10
114	5	20	4.2	44	1.0316	0.595	13.02	3.98	9.04	6.70	0.19	1.45
123	5	26	4.5	55	1.0284	0.594	14.20	5.57	8.63	6.50	0.22	1.21
39	5	30	4.8	80	1.0322	0.582	11.61	2.65	8.96	6.45	0.22	1.60
134*	6	20	3.9	16	1.0317	0.581	13.28	4.31	8.97	6.82	0.09	1.36
80	6	28	4.7	36	1.0281	0.585	14.56	5.83	8.82	6.55	0.12	1.45
130	6	23	4.3	40	1.0310	0.580	13.25	3.99	9.26	5.73	0.21	2.63
11	6	24	3.7	50	1.0285	—	14.15	5.08	9.07	6.50	0.17	1.71
106	6	24	4.4	50	1.0302	0.586	14.02	5.39	8.63	6.50	0.18	1.10
115	6	21	4.3	66	1.0303	0.622	13.80	4.92	8.88	6.45	0.20	1.53
102	6.5	27	5.5	134	1.0303	0.582	14.14	5.34	8.80	6.26	0.14	1.71
12	7	24	4.2	38	1.0286	—	13.86	4.92	8.94	6.60	0.10	1.54
68	7	19	5.2	40	1.0285	0.578	15.46	6.25	9.21	6.50	0.23	1.78
9	7	22	2.9	40	1.0302	—	11.07	2.33	8.74	4.90	0.33	2.83
48	7	35	5.8	70	1.0274	0.574	14.99	6.39	8.60	6.40	0.21	1.28
90	7	28	4.5	70	1.0319	0.572	11.69	2.95	8.74	6.60	0.18	1.25
1	7	33	5.0	80	1.0305	—	13.00	4.12	8.88	7.20	0.13	0.84
88	7	32	5.4	90	1.0303	0.596	13.44	4.52	8.92	6.60	0.16	1.47
124	7	29	5.0	120	1.0317	0.596	13.81	4.21	9.60	6.55	0.21	2.15
104	7	23	5.1	140	1.0313	0.573	12.58	3.80	8.78	6.45	0.20	1.44
101	7.5	30	3.8	28	1.0316	0.577	15.32	5.91	9.41	6.07	0.13	2.53
94	7.5	27	4.9	38	1.0295	0.567	14.63	5.72	8.91	6.35	0.19	1.68
96	7.5	33	4.7	54	1.0337	0.582	12.72	3.44	8.28	6.30	0.19	1.09
6	8	32	3.4	20	1.0294	—	13.31	4.53	8.78	6.76	0.20	1.12
41*	8	29	4.8	10	1.0293	0.583	11.99	3.38	8.61	6.58	0.23	1.24
8	8	21	3.4	30	1.0293	—	12.70	4.08	8.62	6.60	0.23	1.09
34	8	39	4.9	46	1.0308	0.574	11.50	2.93	8.47	6.35	0.17	1.34
89	8	22	5.7	50	1.0300	0.586	14.32	5.16	9.16	6.76	0.20	1.50
116*	8	22	5.2	38	1.0278	0.605	17.00	7.72	9.28	6.55	0.17	1.83
125	8	20	4.9	86	1.0293	0.589	14.35	6.13	8.22	6.50	0.21	0.80
113	8	19	4.1	86	1.0297	0.584	14.14	5.18	8.96	6.50	0.25	1.51
105	8	29	5.6	86	1.0296	0.589	13.86	5.16	8.70	6.55	0.15	1.30
79	8	27	4.6	100	1.0296	0.591	13.08	4.24	8.84	6.35	0.13	1.66
110	8	23	5.5	110	1.0287	0.583	15.21	6.32	8.89	6.44	0.22	1.56
100	8	33	5.4	190	1.0312	0.602	12.53	3.50	9.03	6.40	0.18	1.75
30	8.5	24	4.3	36	1.0281	0.554	15.39	6.44	8.95	6.45	0.25	1.56
127	8.5	33	4.3	46	1.0316	0.590	14.42	5.77	8.65	6.40	0.18	1.33
97	9	23	4.5	60	1.0282	0.596	15.29	7.03	8.26	6.35	0.17	1.04
7	9	25	4.4	70	1.0286	—	12.59	4.22	8.37	5.79	0.22	1.66
49	9	23	5.6	100	1.0281	0.592	14.19	5.65	8.54	6.45	0.19	1.19
92	9.5	18	4.6	36	1.0305	0.558	11.65	3.22	8.43	6.55	0.16	1.01
95	9.5	30	5.9	48	1.0310	0.580	13.13	4.28	8.85	6.50	0.13	1.53
66	9.5	23	6.1	65	1.0306	0.582	16.06	7.25	8.81	6.50	0.26	1.34
128	9.5	30	4.7	66	1.0293	0.590	15.24	5.67	9.57	6.60	0.20	1.63
50	10	21	6.1	20	1.0238	0.569	18.51	10.29	8.22	6.35	0.22	0.94
62	10	20	4.3	56	1.0302	0.565	12.89	4.05	8.84	6.50	0.22	1.42
10	10	20	5.3	90	1.0273	—	13.41	4.97	8.44	6.20	0.17	1.36
81	10	22	5.8	90	1.0317	0.583	12.00	2.86	9.14	6.50	0.16	1.78
43	10	33	5.3	100	1.0289	0.579	13.60	5.01	8.60	6.50	0.14	1.25
67	10	20	5.8	157	1.0318	0.578	13.01	4.07	8.94	6.40	0.25	1.59
93	10.5	33	5.5	30	1.0257	0.568	17.35	9.18	8.17	6.21	0.20	1.06
112	11	21	5.8	66	1.0293	0.600	14.31	5.42	8.89	6.40	0.21	1.59
83	11	22	6.3	70	1.0302	0.600	13.14	4.10	9.04	6.40	0.33	1.52
137	11	33	6.9	94	1.0265	0.576	14.83	5.73	9.10	6.74	0.21	1.45
24	12	28	6.0	32	1.0284	—	11.65	3.45	8.20	6.20	0.16	1.13

Number of Samples.	Age of Child, Weeks.	Age of Mother, Years.	Weight of Child, Kilograms.	Volume of Sample, c.cm.	Specific Gravity, 25°/25°.	Dep. of Freezing Point.	Total Solids, per cent.	Fat, per cent.	Solids, not Fat, per cent.	Lactose anhyd., per cent.	Ash, per cent.	Protein, per cent.
56*	12	26	5.8	16	1.0267	0.550	14.78	6.40	8.38	6.40	0.15	1.12
109	12	30	5.1	52	1.0305	0.590	13.55	4.67	8.88	6.30	0.26	1.53
54	12	21	5.0	52	1.0295	0.573	14.41	5.40	9.01	6.70	0.17	1.43
51	12	27	5.9	54	1.0296	0.580	12.62	3.87	8.75	6.40	0.20	1.45
5	12	23	5.3	60	1.0276	—	15.20	6.28	8.92	6.87	0.18	1.16
23	12	34	5.7	90	1.0259	—	13.46	5.00	8.46	6.35	0.12	1.28
103*	12	22	5.8	46	1.0328	0.587	13.37	3.88	9.49	6.35	0.13	2.23
18	12.5	26	5.0	100	1.0308	—	13.14	4.08	9.06	6.55	0.17	1.65
129	13	27	6.9	44	1.0306	0.597	12.56	3.70	8.86	6.55	0.18	1.44
3	14	20	8.5	34	1.0306	—	14.28	5.18	9.10	6.80	0.17	1.44
28	14	40	5.3	40	1.0279	—	15.11	6.48	8.63	6.45	0.10	1.37
61	14	32	4.0	60	1.0278	0.584	15.33	6.27	9.06	6.45	0.27	1.63
78	14	29	4.8	70	1.0301	0.587	12.22	3.44	8.77	6.99	0.13	0.95
63	14	36	5.4	76	1.0329	0.585	12.28	2.39	9.89	6.82	0.27	2.10
36	14	21	5.8	80	1.0307	0.584	12.65	3.97	8.68	6.50	0.23	1.25
26	14	26	4.8	95	1.0318	—	12.60	3.39	9.21	6.50	0.08	1.95
64	14	24	6.7	168	1.0294	0.580	13.15	4.50	8.65	6.50	0.23	1.22
98	15	33	6.7	36	1.0287	0.586	13.75	4.45	9.30	6.45	0.10	2.06
2	15.5	24	5.5	26	1.0279	—	13.07	4.97	8.10	5.80	0.14	1.47
46	16	24	4.4	24	1.0272	0.556	14.64	5.99	8.66	6.45	0.11	1.43
59	16	25	6.6	30	1.0288	0.573	14.25	6.26	7.99	6.50	0.17	0.60
57	16	23	5.4	38	1.0275	0.580	14.44	5.77	8.67	6.45	0.13	1.39
44	16	40	6.0	44	1.0262	0.556	13.40	5.16	8.24	6.49	0.13	0.92
33	16	23	7.3	110	1.0278	0.553	13.63	5.21	8.42	6.45	0.18	1.09
76	17	31	6.1	54	1.0260	0.577	15.00	6.34	8.66	6.08	0.22	1.66
118	17.5	31	8.7	100	1.0309	0.579	12.45	3.65	8.80	6.50	0.16	1.44
86	18	35	6.1	33	1.0276	—	15.21	6.53	8.68	6.45	0.20	1.33
13	18	24	6.6	50	1.0299	—	12.34	3.50	8.84	6.65	0.13	1.36
52	18	30	6.3	59	1.0293	0.580	14.18	5.23	8.95	6.40	0.15	1.71
32	18	38	7.6	68	1.0308	0.565	12.39	3.53	8.86	6.45	0.20	1.51
15	18	29	7.0	70	1.0266	—	15.58	6.69	8.89	6.25	0.26	1.68
65	18	18	6.2	75	1.0247	0.585	14.05	4.68	9.37	6.45	0.20	2.03
75	19	29	8.1	64	1.0293	0.576	13.42	4.64	8.78	6.26	0.24	1.59
85	19	32	6.1	74	1.0283	0.586	14.51	5.75	8.76	6.55	0.21	1.30
73	20	32	6.8	52	1.0304	0.584	12.10	3.37	8.73	6.30	0.20	1.53
37	20	23	6.4	60	1.0271	0.582	14.93	5.84	9.09	6.45	0.21	1.74
60	20	29	5.9	95	1.0282	0.570	13.09	4.71	8.38	6.40	0.15	1.12
53	20	23	7.2	100	1.0323	0.570	11.33	2.55	8.78	6.60	0.18	1.30
14	21	22	7.3	48	1.0253	—	15.65	7.16	8.49	6.45	0.11	1.22
69	21	23	6.9	74	1.0290	0.584	14.30	5.46	8.84	6.50	0.21	1.43
107	21	37	7.4	100	1.0317	0.575	11.90	3.37	8.53	6.40	0.15	1.28
70	22	22	7.7	30	1.0258	0.545	15.87	7.68	8.19	6.40	0.18	0.90
108	22	24	7.5	32	1.0283	0.597	15.34	7.37	7.97	6.65	0.17	0.44
72	22	24	6.7	35	1.0247	0.562	16.82	8.68	8.14	6.40	0.27	0.77
71	22	26	6.7	80	1.0327	0.597	12.16	2.90	9.26	6.50	0.15	1.92
74	22	26	7.7	130	1.0309	0.592	12.89	3.98	8.91	6.35	0.14	1.72
82	24	22	6.3	36	1.0262	0.560	13.98	5.78	8.20	6.55	0.21	0.72
40	24	25	9.5	60	1.0305	0.562	12.39	3.72	8.67	6.14	0.21	1.62
4	26	28	7.6	80	1.0281	—	13.24	3.90	9.34	7.10	0.09	1.45
77	26	31	9.0	152	1.0288	0.586	13.76	5.25	8.51	6.26	0.18	1.37
55	27	20	7.6	36	1.0284	0.560	14.01	5.43	8.58	6.55	0.12	1.21
31	28	26	7.4	40	1.0271	0.551	15.75	7.60	8.15	6.71	0.20	0.53
29	28	21	8.6	68	1.0274	—	13.74	5.38	8.36	6.40	0.16	1.10
22	28	36	9.5	100	1.0332	—	11.23	2.56	8.67	6.93	0.16	0.87
47	34	43	11.1	124	1.0301	0.587	12.81	4.05	8.76	6.60	0.11	1.40

To summarize the information contained in the above figures the following table has been prepared (Table 2). In this table mean and extreme values are shown for each quantity, and also figures giving the dispersion or variability of each series of measurements.

Three types of mean value are shown: the arithmetic mean, the mode or value round which the results are most closely grouped, and the median or value above and below which equal numbers of the results occur. The upper and lower quartiles, or values between which 25% of the results on either side of the median are to be found, are given also. By dividing the difference between the two quartiles by twice the value of the median the figures given as dispersion have been obtained. By these figures the relative scattering of the different series of results about their median values can be compared.

TABLE 2.

Variability of Composition and Properties of Human Milk after first month of lactation.

	Mean.	Mode.	Median.	Quartiles Upper.	Quartiles Lower.	Max.	Min.	Dispersion.
Volume	66 cc.	40 cc.	58 cc.	84 cc.	40 cc.	168 cc.	10 cc.	0·379
Fat	4·95%	5·30%	4·85%	5·75%	3·86%	10·3%	2·3%	0·195
Ash	0·18%	0·19%	0·18%	0·21%	0·145%	0·33%	0·08%	0·188
Protein	1·41%	1·40%	1·40%	1·58%	1·15%	2·77%	0·41%	0·152
Specific gravity	1·0293	1·0296	1·0294	1·0307	1·0280	1·0337	1·0238	0·063
Total solids	13·74%	13·87%	13·67%	14·53%	12·82%	18·51%	11·07%	0·063
Solids-not-fat	8·79%	8·82%	8·82%	9·00%	8·59%	9·89%	7·97%	0·023
Lactose	6·46%	6·45%	6·49%	6·55%	6·36%	7·20%	4·90%	0·0146
Δ	0·580°C	0·583°C	0·582°C	0·589°C	0·573°C	0·622°C	0·545°C	0·0052
Calories, per 100 gm.	76·9	80·0	75·9					

The various quantities measured have been arranged in the above table in descending order of variability as expressed by the figures for the dispersions.

The most variable quantity is the volume of milk obtained from the breast, for which the dispersion is 0·379. Next come the variabilities of the percentages of fat, ash, and protein, for which the respective dispersions are 0·195, 0·188, and 0·152. These three constituents form a group, the variability of the concentration of each member of which is close to that of the others.

The variabilities of these quantities are about half that of the volume of the sample. The variability of the percentage of total solids and of the difference between the specific gravity of the samples and that of water comes next, with a dispersion of 0·063. The variability of this property is about one-third that of the preceding group. The variabilities of the percentages of solids-not-fat and of lactose follow with dispersions of 0·023 and 0·0146. The last figure is about one-tenth that of the percentages of the other constituents, fat, protein, and ash. Lactose is therefore by far the most constant or least variable of the constituents

measured. The depression of the freezing point, for which the dispersion is 0·0052 has a variability only about one-third of that of the lactose. This quantity is thus the most constant of all those measured in the present investigation.

In the case of each of the measurements, except those of the volume of milk obtained from the breast, and to a less extent of the percentage of fat, the values of the arithmetic, mean, mode, and median lie close together in each series of results. There are considerable differences between the values of the arithmetic mean, mode, and median of the volume of sample for which the figures are respectively 65 cc., 58 cc., and 40 cc. The difference between the mean and the mode amounts to over 50% of the latter. These figures show that there is a preponderance of values above the mode over values below the mode.

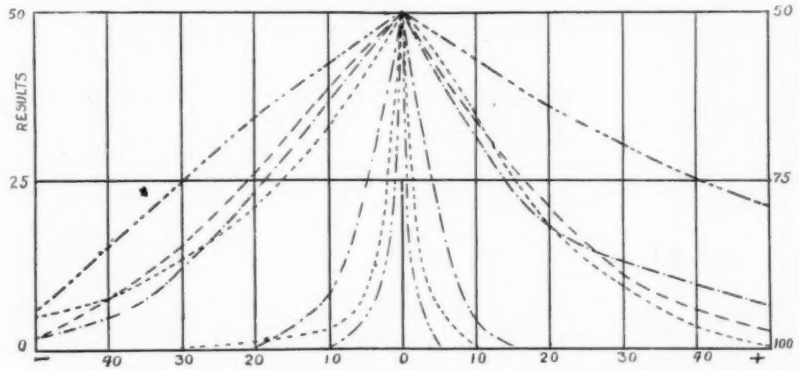

Figure 1.

Variability of human milk: Abscissae: Percentage deviation from median. Ordinates: Percentage of total number of results. Legend (reading downwards): — · — · — volume of sample; ———— percentage of fat; — · — · percentage of ash; – – – – percentage of proteins; — · — · specific gravity; · · · · percentage of lactose; — · · — · · depression of freezing point.

The differences between the mean, mode, and median of the percentages of fat are much less, the figures being respectively 4·95, 5·30, and 4·85. The difference between the mean and the mode in this case amounts to less than 10% of the latter. Here the values greater than the mode only slightly outweigh those below the mode.

The values of the means, modes, and medians of the remaining quantities show no significant differences. These values are: For the percentage of ash, 0·184, 0·190, and 0·181; for the percentage of protein, 1·41, 1·40, and 1·40; for specific gravity, 1·0293, 1·0296, and 1·0294; for percentages of lactose, 6·46, 6·45, and 6·49; for depression of freezing point, 0·580, 0·583, and 0·582. These

figures show that the distribution of values about the mode is approximately symmetrical, and that there is no preponderance of values on one side of the mode over those on the other side.

The values which show the greatest differences between their mean, mode, and median, that is, the volume of the sample and the percentage of fat, and also those which have the greatest variability. The variabilities of these values are not strictly comparable with those of the other quantities measured. The volume of milk obtained from the breast is dependent upon two factors: the volume of milk present in the mammary gland, and the proportion of this which is removed.

The degree of completeness with which the mammary gland is emptied will in turn affect the percentage of fat in the sample obtained. Any variation of the percentage of fat will also affect the specific gravity of the sample, and this property also will be affected by the manner of emptying of the gland.

The percentages of ash, protein, and lactose, on the other hand, and the depression of the freezing point are not, or only slightly, affected by the stage of emptying of the gland at which the sample is taken. These values are determined by factors within the maternal organism, and are not influenced by outside factors.

When the present figures are compared with those given by various authors for the milk of European and American women the ranges of variation of the different constituents are seen to show no significant differences. In this connection reference may be made to the seven series of analyses quoted by Pritchard (10), and the thirteen series quoted by Richmond (11). The average fat content of the present samples seems, however, distinctly higher than that of the figures quoted, which appear to range round the value of about 3·5% On the other hand the percentage of lactose in the present results seems to be lower than the average of many of the series of analyses quoted, which approaches 7·0%. The percentage of protein is also lower than many of the figures given.

In addition to the constituents mentioned above there is the ill-defined group of bodies known as the extractive substances. Camerer and Söldner (*loc. cit.*), who investigated these substances in human milk very thoroughly thirty years ago, found their average concentration ranged from 1% during the first month of lactation to 0·5% towards the end of lactation. Meigs and Marsh (8) give 1% as the average concentration. Denis, Talbot, and Minot (4) investigated the nitrogenous portion of these much more recently. They found it to consist of the same substances (the principal of which is urea) present in the same concentrations as the extractive nitrogenous materials of the blood. Their figures for total extractive nitrogen and urea agree substantially with those of Camerer and Söldner.

The mean, mode, and median of the calorific values of the samples are also given in Table 2. The figures are 76·9, 80·0, and 75·9 large calories per 100 grm.

of milk respectively. The values 9·2, 4·1, and 3·9 have been taken as the calorific values in the body of 1 grm. of fat, protein, and lactose.

When the variabilities of these figures for human milk are compared with corresponding figures for cows' milk (Wardlaw, 18), certain resemblances and differences are observed. As in the case of human milk, the freezing point is the least variable property; the lactose is the least variable constituent. The variability of the percentage of lactose in cows' milk is, however, about three times as great as that in human milk. The ash of cows' milk, on the other hand, the variability of which comes next to that of the lactose, is only about half as variable as the ash of human milk.

The fat and protein in cows' milk have about the same variability as in human milk. The amount of milk obtained at a milking is the most variable property of the samples in the case of cows' milk as well as of human milk, the variability being about the same in the two cases.

It seems rather remarkable that in several respects the milk of individual women is no more, and in some cases considerably less, variable in its properties than the milk of individual cows. It may safely be assumed that the conditions under which the cows lived were much more uniform than the conditions under which the women lived. In addition, the dairy cow is the result of a long period of selective breeding for the purpose of improving milk production. It would appear that the improved average milk production which can undoubtedly be brought about by this selection is not necessarily accompanied by a decreased variability of the product of the individual animals. Breeding has been successful in producing animals whose milk does not as a rule fall below certain specified values in certain respects, rather than a product whose properties converge to these values.

The relative variabilities of these results are also shown graphically (figure 1). In the figure the abscissae represent the percentage deviations of the different quantities from the median values. The scale for ordinates to the left of the median abscissa runs upwards; the scale for ordinates to the right of the median abscissa runs downwards. The curves obtained in this way are simply ogival curves, in which the ordinates have been measured in the usual way below the median, but in the reverse direction above the median. Instead of the usual S-shaped ogival curves, those obtained rise to a peak at the median. The steepness with which the peak is reached is a measure of the variability or dispersion of the results. The sharper the peak is, the less is the dispersion. The relative variabilities are more readily seen when the curves are plotted in this way than when they are in the ordinary ogival form.

These curves also avoid certain difficulties which are encountered when the variabilities are shown graphically by the frequency or probability curves plotted respectively by arranging the results in arbitrary groups, or by drawing the derived curves of the ogives.

Relation between Stage of Lactation and Properties of Milk.

In the following table the average values of the properties and composition of the samples obtained during different periods of lactation are shown. Four periods have been selected, so as to give groups containing comparable numbers of samples: from 3 to less than 8 weeks, from 8 to less than 12 weeks, from 12 to less than 16 weeks, and from 16 to 34 weeks. The first three groups cover monthly intervals (only one specimen occurring in the 3 weeks period). The last group covers a much longer interval.

TABLE 3.

Variation of Human Milk with Stage of Lactation.

Age of child (weeks) ..	3–7·5	8–11·5	12–15·5	16–34
Age of mother	27·5	25·5	27·6	27·5
Weight of child (kgm.) ..	4·36	5·15	5·75	7·14
Volume of sample (c.cm.) ..	62·7	71·0	63·3	66·7
Specific gravity (25°C./25°C.)	1·0302	1·0292	1·0295	1·0286
Depression of freezing point	0·585°C.	0·583°C.	0·581°C.	0·573°C.
Fat (per cent.)	4·66	5·29	4·54	5·17
Lactose anhydride (per cent.)	6·41	6·45	6·51	6·48
Ash (per cent.)	0·18	0·20	0·18	0·17
Protein (per cent.)	1·55	1·42	1·43	1·35
Number of samples, total ..	33	31	20	37

The variation of the average ages of the mothers of the above groups is only slight. This factor therefore introduces no complication into the variation due to the average ages of the children.

The average weights of the infants increase in a normal manner. The figures shown agree closely with the average figures for the two sexes given by Robertson (12) for South Australian infants of the same average ages.

The average volume of milk obtained from the breast shows no regular movement with the stage of lactation, but fluctuates slightly about the mean figure for the whole series of results.

The average specific gravities show a similar fluctuation about the mean value. But in this case the fluctuations are in the opposite direction to those of the volume.

The average values for the depression of freezing point fall as the stage of lactation becomes later. This fall is very slight between the third and sixteenth week of lactation, but more rapid between this period and the next. The range of variation, however, is only slightly over 1% of the mean value.

The average percentages of total solids and of fat fluctuate about the mean

value in the same way as the average volumes of the samples, larger volumes being associated with higher percentages of total solids and of fat.

The average percentages of solids-not-fat fluctuate about the mean value in the opposite direction to the percentages of fat.

The average percentages of lactose show a rise as the period of lactation increases up to the sixteenth week, then a slight fall between this and the last period.

The figures of Denis and Talbot (4) show a much greater rise in the percentage of lactose during the course of lactation, and also higher figures than those of the present results. Many of their figures for the percentage of lactose exceed 8% after the tenth week of lactation.

From the end of the first month of lactation the percentage of ash varies in the opposite direction to the percentage of lactose.

The percentage of extractives shows only a slight tendency to fall during the period of lactation studied. The figures (calculated as described above) are as follow:

Week of lactation	4–8	8–12	12–16	16–34
Extractives (per cent.) ..	0·78	0·72	0·68	0·72

The values show little variation from the average for the whole period, which average is close to that observed by Camerer and Söldner (1) (*loc. cit.*).

The average percentages of protein fall slightly during the period of lactation shown in the above table. When these figures are considered in conjunction with those for the earlier stages of lactation (Wardlaw, 16, *loc. cit.*), the manner of variation of protein content with stage of lactation is shown more clearly. In the following table (Table 4) the average percentages of protein are shown from the first day of lactation to about the twenty-fifth week. The corresponding average weights of the infants are also shown where available.

TABLE 4.

Relation between Age of Child, Weight, and Protein in Milk.

Age.	Samples.	Body Weight. (kilograms).	Protein (per cent.).
0	—	3·56	—
1–2 days	3	—	3·30
3–4 „	39	—	2·33
5–7 „	36	—	2·06
7–11 „	24	—	1·86
3–7 weeks	33	4·36	1·61
8–11 „	31	5·15	1·42
12–15 „	20	5·75	1·43
16–34 „	37	7·14	1·35

The figures in the above table are plotted in the accompanying curves (Fig. 2). Periods of lactation are plotted as abscissae, while the percentages of protein and weights of the infants are plotted as ordinates.

The curve for the percentages of protein shows clearly the very rapid fall in the concentration of this constituent during the first week of lactation. During this period the values drop from over 3·3% to 1·86%. During the next five weeks the fall is less rapid, the value reached at the end of this period being 1·61%.

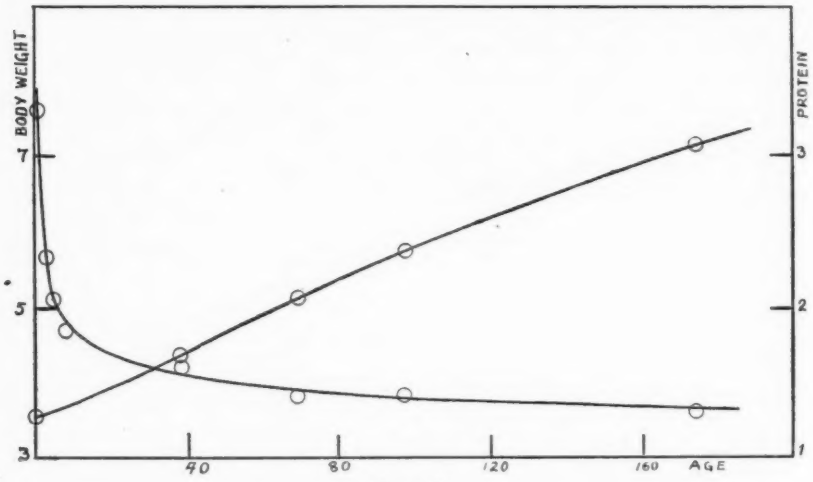

Figure 2.

Relation between percentage of protein in human milk and age of child (days), and between body weight (kg.) and age of child.

From this stage on the fall in the concentration is very slow. In the period between six weeks and thirty weeks the value decreases only from 1·61% to 1·35%. The percentage of protein may be said to reach a steady state after the first month of lactation.

The results differ from those obtained by one of us (Wardlaw, 18, *loc. cit.*) on the milk of individual cows. In the case of these animals the percentage of protein, after falling sharply during the early stages of lactation, rose again steadily, so that the concentration of protein was about 30% higher at the end of the thirty-sixth week of lactation than it was at the end of the eighth.

Variation with Age of Mother.

The following table shows the variations in the average properties of the samples of milk obtained from women between different ages. The ages have

been placed in four groups, selected so as to give groups of samples comparable in number.

TABLE 5.

Variation of Human Milk with Age of Mother.

Age of mother (years) ..	18–22	23–26	27–31	32–43
Age of child (weeks) ..	10·9	13·8	12·1	12·0
Weight of child (kgm.) ..	5·32	5·65	5·76	5·63
Volume of sample (c.cm.) ..	60·7	65·6	67·0	71·2
Specific gravity (25°/25°C.)	1·0291	1·0291	1·0296	1·0297
Depression of freezing point	0·582°C.	0·587°C.	0·582°C.	0·582°C.
Total solids (per cent.) ..	13·95	13·98	13·51	13·45
Fat (per cent.)	5·13	5·30	4·65	4·63
Solids-not-fat (per cent.) ..	8·81	8·70	8·86	8·78
Lactose anhydride (per cent.)	6·45	6·44	6·47	6·50
Ash (per cent.)	0·19	0·18	0·17	0·17
Protein (per cent.)	1·51	1·37	1·48	1·41
Number of results, total ..	30	35	28	30

The average ages and weights of the children belonging to the women of the different age groups do not vary enough to introduce complications due to variations of stage of lactation.

The average volume of the sample increases distinctly as the average age of the subject increases. It must be borne in mind, however, that as the average age of the groups increases the proportion of primiparae which they contain decreases. It is usually more difficult to empty the breast by manual expression in the case of primparae than in multiparae. This circumstance might enable larger average samples to be obtained from multiparae of the older age groups, even if their milk secretion were not increased.

If the increase of the average volumes of the samples from the older age groups be due solely to more complete emptying of their breasts, a corresponding variation of the percentage of fat should be apparent. The fat content of the portions of milk last expressed from the breast are always higher than that of the preceding portions. The figures, however, show no parallel variation of the average volume and fat content, and give no indications that the difference is due to variations and the degree of emptying of the breast.

The average specific gravity is distinctly higher for the two older groups than for the two younger.

The average depression of freezing point is distinctly higher for the group between the ages of 23 and 26 than for the other groups.

The percentage of total solids varies in a direction opposite to that of the

specific gravity; it is higher for the two younger groups and lower for the older groups.

The average percentage of fat varies in the same direction as the specific gravity, and is the cause of the variation of the latter. The milk of the two younger ages is distinctly richer in fat than that of the two older groups.

The average percentage of solids-not-fat does not vary in one direction, but fluctuates about the mean value for the whole series.

The average percentages of lactose and of protein also show no definite trend in one direction, but fluctuate about the mean values.

Relation between Number of Children Suckled and Composition of Milk.

In general it may be said that there is no obvious correlation between the ages of the women and the properties of their milk, other than that of the volume obtained from the breast. This increases with average age. There does not appear to be any very cogent reason for expecting any definite correlation between the properties of the milk and the age of the women except towards the extremes of the period during which lactation is possible. One would be more inclined to expect the mammary gland to reach and maintain a certain capability during this period.

When the properties of the samples are compared, not according to the ages of the mothers, but according to the numbers of times which they have suckled children, a relation appears between the number of children suckled and the volume of milk obtained from the breast, and also between the number of children and the weight of fat in the milk obtained. This is shown in the following table:

TABLE 6.

Average Volumes of Milk and Weights of Fat obtained from a breast of women who have suckled different numbers of children.

No. of Children Suckled	No. of Samples.	Age of Mother.	Volume of Sample.	Per cent. Fat.	Weight Fat.
1	65	24·7	63·7	5·16	3·29
2	24	26·8	66·7	4·84	3·26
3	17	30·4	71·3	4·68	3·34
4 and over	15	33·5	72·7	4·69	3·41

The average volume of the sample obtained from the breast increases with the number of children suckled by the subject. This increase is rapid at first, up to the third child suckled, and then becomes slower. The figures are plotted in the accompanying diagram (figure 3). This diagram reveals a tendency on

the part of the volumes to approach a constant value in women who have suckled over four children.

These figures are in harmony with the view of MacFarlend (7), that a new set of acini develops in the mammary gland at each successive pregnancy. The constant volume which appears to be approached after a certain number of pregnancies is possibly that secreted when all the potential acini are actually secreting.

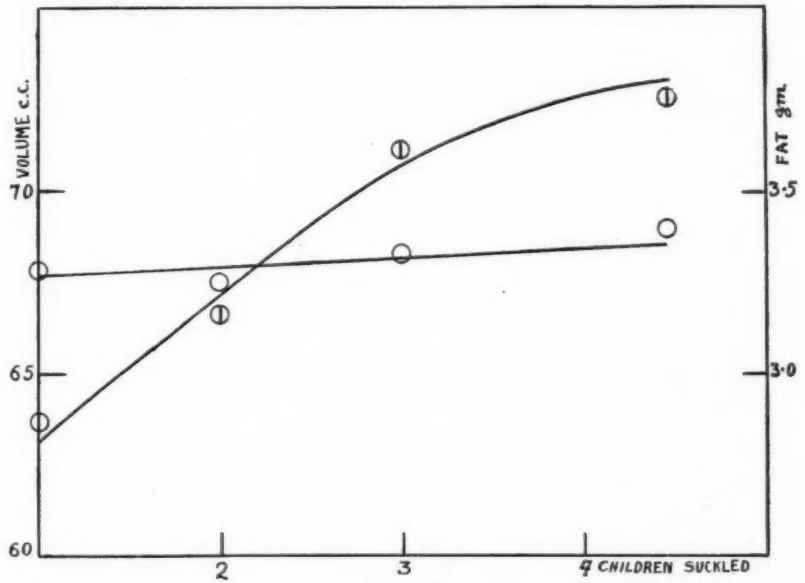

Figure 3.

Relation between number of children suckled, average volumes of milk obtained from breast Φ, and average weights of fat in the milk obtained O.

The figures for the weights of fat contained in each breastful of milk show quite a different variation with the number of children the women have suckled. There appears to be a very slight steady increase in the amount of fat in the samples of milk. This increase is so slight, however, that the weight of fat obtained from the breast can be said to show no material variation with the number of children suckled.

In other words, women tend to yield greater volumes of milk poorer in fat as the number of children suckled increases. The decrease in the percentage of fat is related to the volume of milk obtained in such a way that the weight of fat obtained is practically constant. The percentage of the substances other than fat shows no evident dependence on the number of children suckled.

Relation between Percentage of Ash and Percentage of Lactose.

Jackson and Rothera (6) have shown that there is an inverse relationship between the electrical conductivity of human and other milk and the percentage of lactose. A similar relationship should therefore exist between the concentration of ionized salts and the percentage of lactose, since the conductivity is determined by the former. The relationship will be modified by the fact that the conductivity is depressed by the protein content of the milk to the extent of nearly 3% for each 1% of protein present.

Figures for the concentration of ionized salts are not available for the present results, but when the average percentages of total salts or ash are compared with the corresponding percentages of lactose a similar inverse relationship is evident. This is shown in the accompanying table.

TABLE 7.

Relationship between Ash, Lactose, and Delta of Human Milk.

Lactose.	Lactose, Mean.	Ash, Mean.	△, Mean.	No. of Samples.
6·2% and under	5·84	0·205%	0·577°C.	11
6·2 6·45	6·38	0·186	0·580	50
6·45 6·7	6·55	0·179	0·582	45
Over 6·7	6·85	0·158	0·578	15

The similar relation which is to be seen when the average percentages of lactose corresponding to different percentages of ash are compared, is shown by the following figures:

Percentage of Ash	under 0·15	0·15–0·20	0·20–0·25	0·30 and over
Average % Lactose	6·68	6·51	6·40	6·28

The values of △ have also been recorded in Table 7. They show no significant variation with the percentage of ash or of lactose, and demonstrate that variations in the concentration of lactose must be compensated for by inverse variations of the concentrations of soluble salts and other soluble matter.

When the percentages of ash and of lactose for the individual milks are compared the inverse relationship between the two is not shown so clearly as in the above table. This is no doubt due to the fact that the proportion of soluble to total ash varies considerably in the individual specimens (Michaelis and Rona, 9; Wardlaw, 19). In the average figures, on the other hand, the variations of the proportion of soluble ash in one direction are approximately compensated for by variations in the other direction.

Relation between Disturbances of Alimentary Canal and Composition of Milk.

The women whose milk has been examined in this investigation were in every case chosen as showing no material departure from normal, and giving no history of any recent serious illness. They were selected as suckling children who were also normal as far as could be determined, and for which no history of any serious illness was obtained.

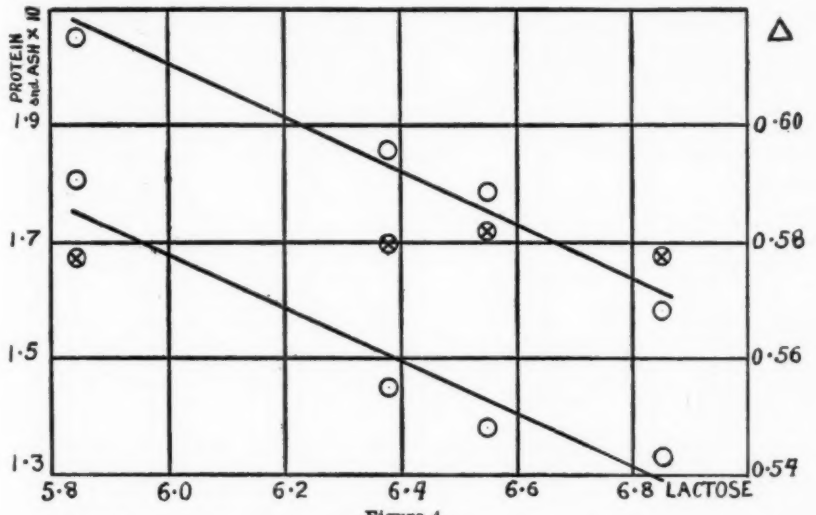

Figure 4.

Relation between percentage of lactose in human milk and percentages of ash (upper curve), percentage of protein (lower curve), and Δ (②)

In certain of the histories, however, reference was noticed to the child having had "diarrhoea and green motions." Six cases of this kind occur. The composition of the mothers' milk in these cases, and the ages of the mother and child, and the weight of the child are shown in the following table:

TABLE 8.

Relation between Composition of Mother's Milk and Alimentary Disturbance in Infant.

No.	Age of Mother. yr.	Age of Child. wk.	Wt. of Child. kg.	Sample Vol. of. cc.	Fat %	Protein %	Lactose %	Ash %
9	22	7	2·9	40	2·33	2·35	4·90	0·33
25	32	3	2·6	46	4·8	2·15	5·13	0·21
26	26	14	4·8	95	3·39	1·77	6·50	0·08
27	29	5	3·7	40	6·61	1·72	6·35	0·12
37	23	20	6·4	60	5·84	1·63	6·45	0·21
100	33	8	5·4	190	3·50	1·64	6·40	0·18
Mean	27·5	9·5	4·3	78·5	4·4	1·86	5·95	0·19

In each case the percentage of protein is above the average. The weights of the children are below the average, except numbers 37 and 100, the two lowest proteins of the series. The figures for lactose show a variation in the opposite direction to the figures for the protein.

Two of the above specimens (26 and 29) show exceptionally low percentages of ash, while two others (9 and 25) show exceptionally low percentages of lactose. The two latter specimens are those containing the two highest percentages of protein in the series. The first of them contains also an exceptionally high percentage of ash.

These figures are in general harmony with a widespread opinion that excess of protein in the mother's milk may be associated with alimentary disturbances in the infant. Carter and Richmond (2) many years ago published analyses showing this association. It should be observed, however, that in the four of the above cases in which the infants were under weight the mother's milk showed extreme variations in constituents other than the protein, and that many instances occur of percentages of protein as high as some of those in the above table in cases where there is no record of alimentary disturbance in the infant.

In conclusion, we wish to express our indebtedness to the staff of the Newtown Baby Clinic, without whose enthusiastic co-operation the bulk of this work would have been impossible. We are indebted also to the staff of the Tressilian Hospital, through whose good offices specimens of milk were obtained from several patients.

We also wish to express our thanks to Professor H. G. Chapman, in whose laboratory this work was done, for the interest he has shown in the investigation.

SUMMARY.

The modal values for the composition and certain properties of the milk of Australian women obtained between the first and ninth months of lactation are as follows: Depression of freezing point, 0·0583°C.; lactose, 6·45%; specific gravity, 1·0296; protein, 1·40%; ash, 0·19%; fat, 5·30%. These figures are in ascending order of the variability or dispersion of the quantities, which range from 0·0052 for the depression of freezing point to 0·195 for the percentage of fat.

The average percentages of protein and of ash, and the depression of freezing point, show a slight fall as lactation continues. The average percentage of lactose shows a slight rise.

The milk of the younger women is on the average distinctly richer in fat than that of the older women. The average volume of milk obtained from the breast varies in the opposite direction with age.

The average percentage of ash is slightly greater in the milk of the younger women, while the average percentage of lactose varies in the opposite direction.

The milk of the women between the ages of 22 and 27 has a distinctly greater average of depression of freezing point and smaller average percentage of protein than in the case of other age groups.

The average volume of milk obtained from the breast increases with the number of lactations up to the fourth. The average percentage of fat decreases, so that the average weight of fat obtained remains constant.

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OBSERVATIONS ON MITOCHONDRIA

by

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(Submitted for publication, 5th August, 1926.)

In this paper a further series of observations are reported concerning mitochondria of certain protozoa, which may help to elucidate the nature of these cytoplasmic inclusions.

VITAL STAINING OF MITOCHONDRIA.

Experiments have been carried out on a new dye stuff, the sodium salt of diethyl safranine monocarboxylic acid, prepared from janus green by the hydrolysis of the nitrile, and kindly forwarded to me by Professor T. Brailsford Robertson. Dilute solutions (1 part in 5,000) have the colour of weak permanganate. With this substance, and at a dilution considerably greater than this, I have succeeded in staining the mitochondria in living paramoecia, and in an unidentified heterotrichan. The mitochondria are coloured a bright pink in about twenty minutes. In living amoebae they colour only very faintly, the organism encysting before the stain has had time to penetrate extensively. A heterotrichan in which the mitochondria have been thus rendered visible is shown in figure 1.

The stain does not appear to have any corresponding selective action on bacteria. This observation is of special importance, in view of the work of Wallin (9), who reports having succeeded in staining various living bacteria with janus green dilutions of 1 to 10,000. Tubercle bacilli stained very faintly, but certain unidentified bacilli and cocci were very intensely stained immediately the preparation was made.

In the parasite of Rocky Mountain spotted fever, described by Wellbach, Cowdry states, on the contrary, that symbiotic bacteria can be seen lying side by side with the mitochondria.

The stain employed in this investigation does not, however, lend any support to Wallin's contention. I find that in a culture of heterotrichan infusions, at the time when the mitochondria have been coloured a bright pink, bacteria may be observed in the surrounding fluid quite unstained, and still exhibiting active

movement. It might be urged that these bacteria are covered with an impermeable membrane, which is absent in the mitochondria (assuming them to be symbiotic bacteria); but this seems to be disproved by the following observations. Mobile bacilli, after having been exposed for some hours to the stain, die; it seems justifiable to contend that the stain has penetrated them; nevertheless, they are not selectively coloured by it. I have tested this dye against the following bacteria, and after an exposure of six hours, and even long after it has killed them, it has not rendered them stainable: Bacillus coli communis, staphylococcus albus, and staphylococcus aureus.

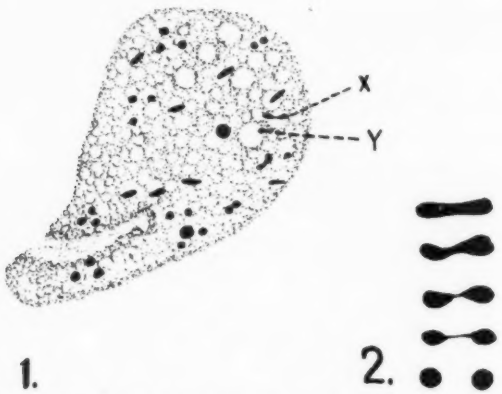

Fig. 1. A Heterotrichan Infusion showing mitochondria stained alive by "Janus red." Two of the mitochondria, indicated by X and Y, are seen undergoing binary fission. Some of the mitochondria are in the rod-shaped condition, others in the spherical.

Fig. 2. Various stages in the binary fission of the mitochondrium, indicated by X in the previous figure. The drawing was made from a mitochondrium which had absorbed the stain, and the process occupied four minutes.

There is a further difference between ordinary bacteria and mitochondria, namely, that bacteria exposed to absolute alcohol for several hours are still sharply stainable with iron haematoxylin, while mitochondria are rendered non-stainable, even after quite a short exposure. That mitochondria have a bacterial appearance cannot be questioned, but it is equally certain that they are not ordinary bacteria. That "mitochondria are symbiotic bacteria in the cytoplasm of all higher organisms, whose symbiotic existence had its inception at the dawn of phylogenetic evolution," as suggested by Wallin, cannot in the present state of knowledge be profitably discussed, and that they may be bacteria which have become incorporated into the evolving cell in a manner reminiscent of that developed in Mereschkowsky's and Minchin's speculation concerning cell evolution (6) is also at present beyond the bounds of scientific investigation. Whatever their origin, however, it seems very certain that they cannot at the present day be bacteria, for they now resemble bacteria only in external appearance.

Fission and Fusion of Mitochondria.

If mitochondria are examined in fixed and stained preparations, numerous conditions of these bodies are observed, which seem to indicate that they are undergoing binary fission. This was apparently first observed by Faure-Fremiet in certain protozoa. I have observed the same thing in Paramoecium (4) and Opalina (3), while M. R. and W. H. Lewis have figured apparently similar conditions in mitochondria observed in cells grown in tissue culture, and the occurrence of fission in mitochondria seems to be widely accepted at the present day. Similarly the occurrence of multiple fission in the long, filamentous mitochondria in the actively secreting pancreas cells seems clearly established. But the actual process of fission has not, so far as I am aware, ever been observed in living mitochondria.

In this paper I am able to report observation in which I have been able to follow the process of binary fission in living mitochondria. The observations nave been made on mitochondria from a heterotrichan infusian stained *intra vitam* by the dye described above, and also on living unstained mitochondria from amoeba. The process was first seen in the two mitochondria indicated by X and Y in the heterotrichan shown in figure 1.

Different stages in the fission of this mitochondrium are shown at higher magnification in figure 2. The rod-shaped mitochondrium contracts slightly in the middle; the constriction then slowly becomes more marked, until finally two almost spherical bodies are formed, connected to one another by a thin filament; the filament then ruptures, and the two daughter forms round themselves off and become spherical. The whole process lasts four minutes.

In amoeba I have been able to observe fission. Since the observations have had to be made on entirely unstained specimens, the process has not been followed out with the same clearness as I have seen it in stained heterotrichan mitochondria. Nevertheless, of the actual occurrence of binary fission of rod-shaped mitochondria in other forms there can be no doubt. The best time to observe it is in the encysting forms of amoeba. An amoeba may be readily made to undergo encystment by placing it in an environment unfavourable to it, such as a weak janus green solution. Although the janus green does not extensively penetrate the organism, yet the latter rounds itself off, and in such forms numerous spherical bodies in pairs, and also many dumb-bell shaped forms, may be seen, evidently indicating an extensive occurrence of binary fission.

Fission of mitochondria accompanying encystment of the organism apparently occurs, therefore, in amoeba as it does in Opalina (3) and in Paramoecium (4).

Not only fission, but also fusion of mitochondria can be demonstrated in protozoa. An increase in size of mitochondria has been described by J. T. Scott (8); and Cowdry (2) believes that increase in size may result through a

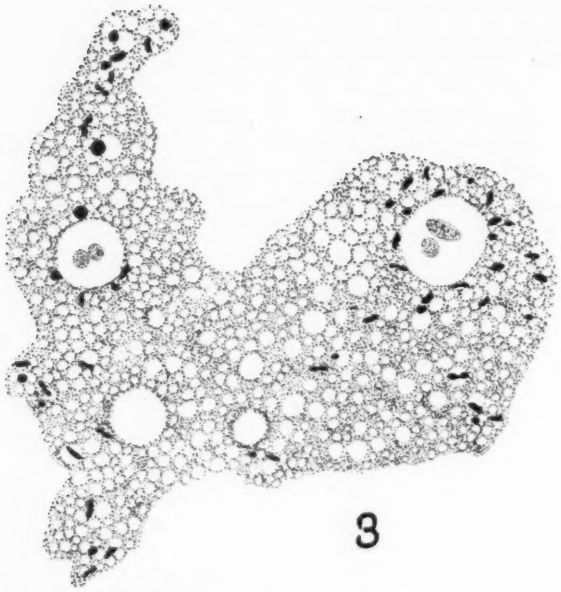

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Fig. 3. Living amoeba, showing mitochondria. Most of the mitochondria are in the rod-shaped condition; several are spherical. Two of the vacuoles contain food, but not mitochondria—they have evidently already dissolved.

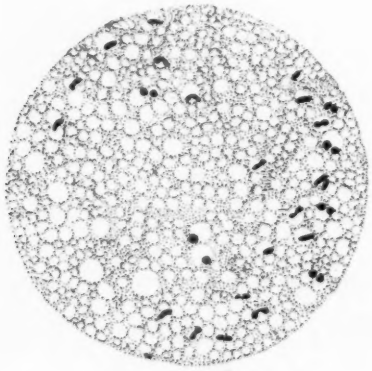

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Fig 4. An encysting amoeba, showing numerous mitochondria which are undergoing, or have just undergone, binary fission.

coalescence of two or more mitochondria. Similarly, W. J. M. Scott (7) has described the formation in the pancreas after phosphorus poisoning of large lipoid droplets produced apparently by fusion of mitochondria. I have also previously reported fusion of mitochondria in pancreatic cells, in cases when the pancreas has not been fixed immediately after killing the animal; and a similar fusion of mitochondria may be observed in opalina in cases when the organism has been allowed to live in Ringer's fluid for about twelve hours.

As, however, actual fusion of mitochondria has not been demonstrated in these cases, I may report an observation made on living amoeba, in which I have actually seen such fusion. In living amoebae many of the mitochondria are rod-shaped bodies; but frequently spherical forms also occur (such, for example, in figure 7 r). In the streaming cytoplasm of the living amoeba these highly refractive bodies are easily recognizable; they exhibit marked Brownian movement, and are constantly being swept about in the currents of protoplasm. On several occasions I have observed two such mitochondria always in the spherical condition, lying close together, and suddenly fusing with one another (see, for instance, figure 7 r). The fusion is probably a surface tension effect, similar to the fusion of oil globules, that may be observed in any suspension of oil in water, and it does not seem likely that any physiological function can be ascribed to it.

The Rôle of the Mitochondria in the Process of Intracellular Digestion. Observations on Living Amoebae.

Living amoebae are particularly favourable objects for the study of the behaviour of mitochondria, since these bodies are clearly seen as highly refractive bodies in the granular cytoplasm of the organism, and moreover, the movements undergone by the organism are so slow as to permit of leisurely observation.

In a previous paper (4) I have recorded an observation made on fixed and stained paramoecium, in which the food vacuoles were observed to contain rod-like bodies, similar to mitochondria in appearance, and which were not bacteria. These bodies gradually dissolve in the fluid contents of the vacuole, and since mitochondria are believed to be of an enzymatic nature, it was concluded that the process of extrusion of mitochondria into the food vacuoles constituted a visible passage of the digestive enzymes into the digestive vacuoles. We would here have a process of intracellular digestion, analogous to the process of extracellular digestion seen in ordinary glands, where the enzymes likewise appear to arise from the mitochondria.

In living amoebae I have been able to follow the whole processes of vacuole formation, and the relation of mitochondria to the process. When a foreign organism is engulfed by a living amoeba, the body circulates in the cytoplasm of the organism for a considerable time, lying in direct contact with the protoplasm,

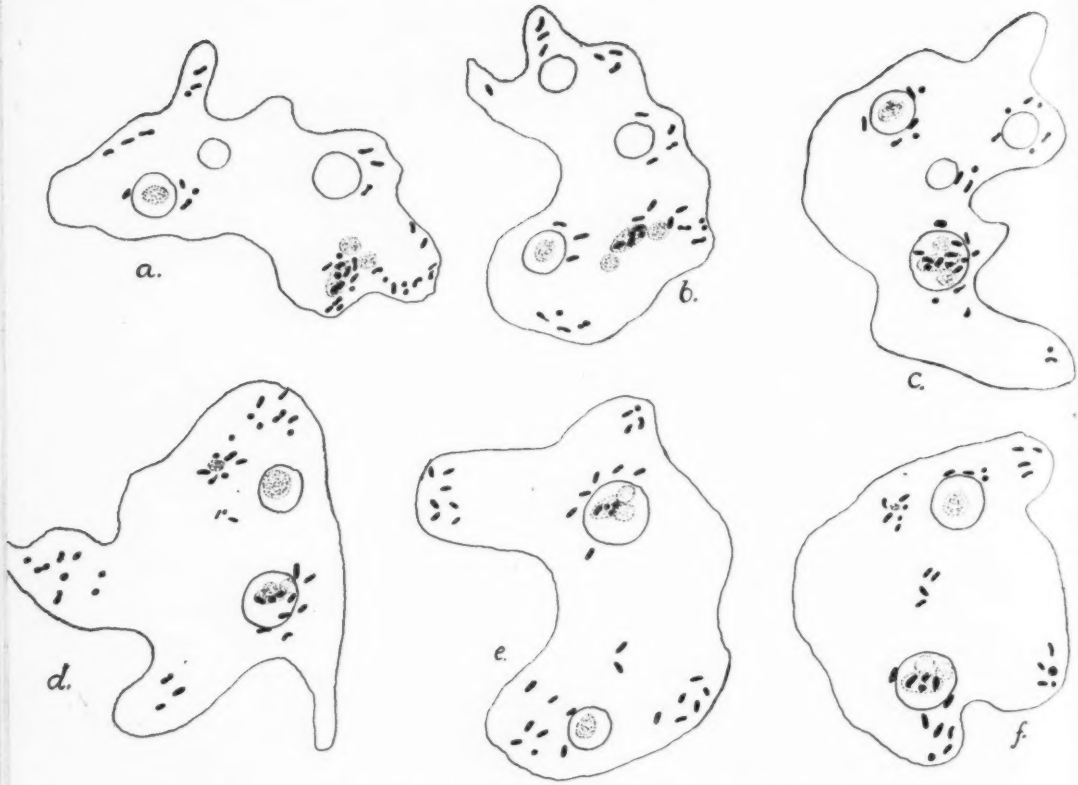

Fig. 5. Drawings of a living amoeba, taken at various times, showing the behaviour of the mitochondria, and their relation to the process of digestion. The time occupied in these changes was not sufficient to permit representation of all the mitochondria accurately in their correct situation. (a) 7.55 p.m. Numerous rod-shaped mitochondria are scattered about in the cytoplasm. Three ingested food particles are seen, to one of which four mitochondria adhere. Two excretory vacuoles and one food vacuole are observed. (b) 8.5 p.m. One of the mitochondria on the surface of the ingested food is dividing. (c) 8.15 p.m. A vacuole has formed around the three particles of ingested food. Five mitochondria adhere to one of the food particles (the remaining mitochondria which appear to lie within the vacuole are really above it within the cytoplasm). (d) 8.25 p.m. Another very small food particle has been engulfed, and is surrounded by several mitochondria. Its fate has not been followed out in the remaining figures. (e) 8.35 p.m. (f) 8.50 p.m.

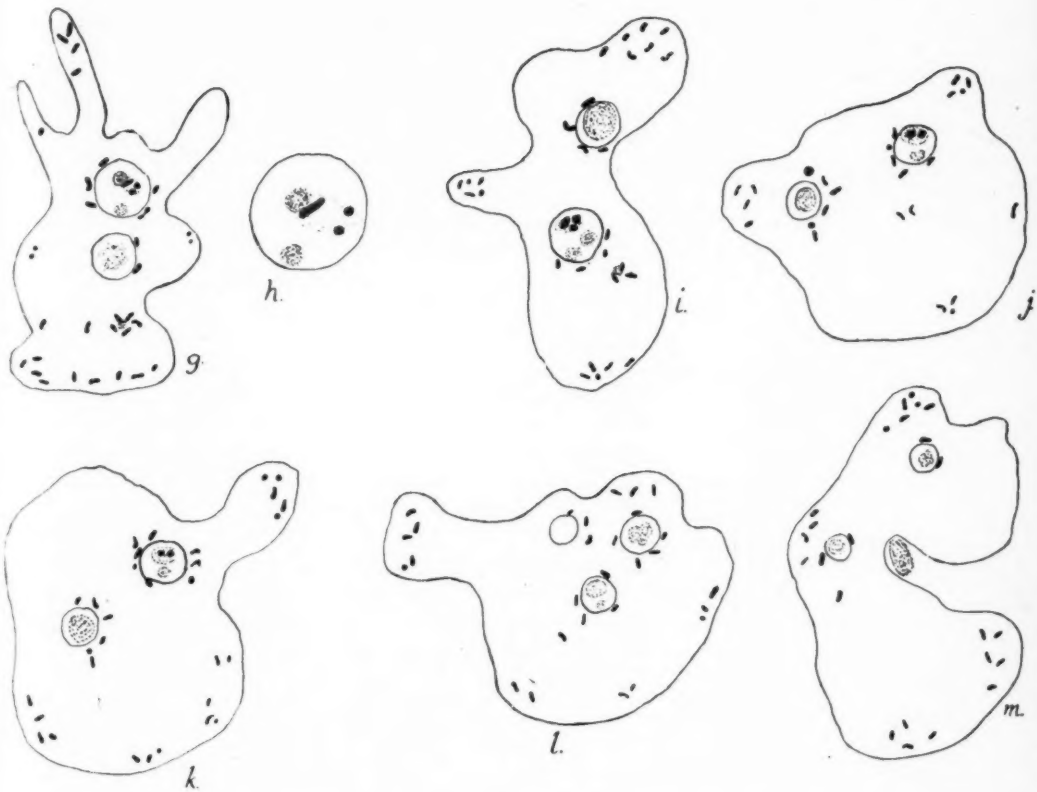

Fig. 6. (g) 9.15 p.m. Two of the mitochondria within the vacuoles have become spherical. (h) Food vacuole shown in the last figure at higher magnification. The spherical shape of the two mitochondria is very clearly seen. (i) 9.20 p.m. The mitochondria within the vacuole have rounded themselves off. (j) 9.35 p.m. One mitochondrium has been dissolved within the vacuole, and the vacuole has decreased in size. One of the particles of food has disintegrated and entirely disappeared. (k) 9.45 p.m. The mitochondria within the vacuole have decreased in size. (l) 9.55 p.m. The mitochondria have entirely dissolved and the vacuole has diminished greatly is size. (m) 10.16 p.m. One food particle has entirely disappeared, and the vacuole has decreased in size. The amoeba is at the same time engaged in engulfing a foreign organism (Euglena).

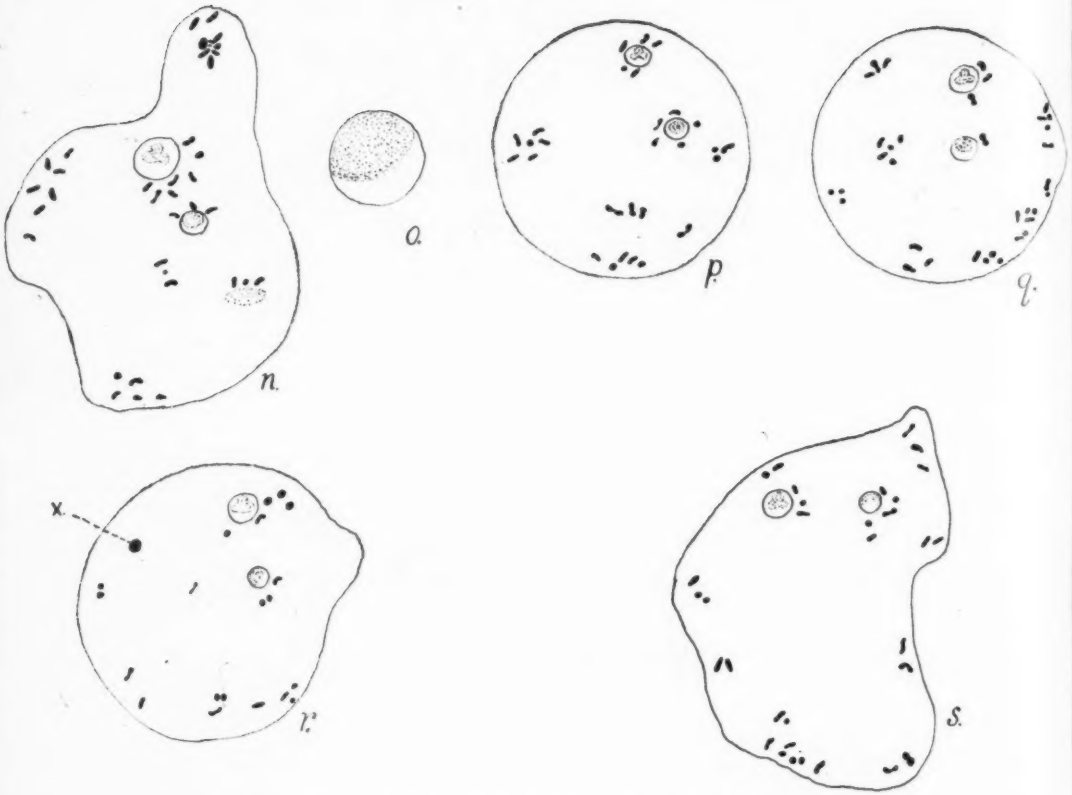

Fig. 7. (n) 10.30 p.m. The vacuole has decreased greatly in size; the Euglena has become ingested, but the vacuole has not yet been formed around it. Time did not permit the following up of this food particle. (o) The same food vacuole as is shown in the previous figure, at a higher magnification. Note the great dimunition in size of the vacuole in relation to the size of the ingested food. (p) 10.55 p.m. The amoeba underwent a temporary encystment; numerous mitochondria are seen undergoing binary fission. (q) 11.10 p.m. Amoeba emerging from cyst. (r) 11.15 a.m. A large mitochondrial body has been formed by the fusion of at least two (indicated by X). (s) 2 a.m. The food particle has not even yet been fully digested; possibly it contains indigestible material. At this stage the observation was stopped.

and not being separated from it by any visible vacuole. As this engulfed food moves about within the amoeba through the action of the streaming currents of protoplasm, it comes in close relation, frequently with the mitochondria which are scattered about in the organism. These mitochondria adhere to the engulfed prey. I have also seen cases where a narrow current of protoplasm moves past a stationary engulfed organism, and have seen a mitochondrium carried to the organism by this protoplasmic stream, and then observed the mitochondrium to adhere to the food, while the current swept past. By this means a small number of mitochondria come to adhere to the engulfed prey (see, for example, figure 5, a, b). It is, in fact, a common sight in living amoebae to observe an engulfed ciliate carried about in the protoplasmic currents, having attached to its surface a number of refractive bodies, the mitochondria. In this situation it may at times be observed that the mitochondria actually undergo fusion, and at other times fission.

After a number of mitochondria have adhered, probably merely through chance, to the surface of the food, a vacuole is gradually formed around the food particles. This condition is shown in figure 5 c, where at least five mitochondria are attached to the large food particle, and this food particle and two smaller ones are now contained in a single large vacuole. These vacuoles then slowly circulate about in the streaming protoplasm. At times the mitochondria may be observed to undergo binary fission within the vacuoles. After about half an hour the mitochondria suddenly disappear within the vacuole, having evidently become dissolved in the fluid contents of the vacuole (figure 6 l). The food at the same time seems gradually to disintegrate, and the vacuoles become very markedly reduced in size. The disintegration of the food is difficult to observe, and I have not been able to follow it to completion; but the reduction in size of the vacuole is very clearly seen, indicating probably an absorption of digested food (see figures 5, 6, and 7, l, m, n, o, p, q, r).

In a previous paper I described a preparation of paramoecium, in which a mitochondrium seemed to be actually passing through the wall of the vacuole. Whether this preparation really represented the entrance of a mitochondrium into the vacuole is, however, difficult to say. In amoeba I have never observed the extrusion of mitochondria into the vacuoles, but the mitochondria always adhere to the food particles first, and it is only subsequently that the vacuole is formed around the food particle. There seems to be good evidence in amoeba that the mitochondria cannot penetrate the membrane of the vacuole. In living amoebae, especially those with very large vacuoles, I have frequently seen mitochondria lying in the cytoplasm close against the walls of the vacuole. Here they always undergo typical Brownian movements; they may even indent the wall of the vacuole, but they never seem to be able to penetrate it. I have made this observation on various occasions, and have observed numerous such mitochondria lying closely against the vacuoles, but on no occasion have I ever seen them actually penetrate into their interior.

On the Number of Mitochondria which occur in Single Cells.

The statement has occasionally been made that the number of mitochondria are approximately constant within similar cells of similar species. For example, Wakelin Barratt (1) states that in the liver of the rabbit "The number of chondriosomes present in a single cell is approximately 70, the variations met with not appearing to be considerable." I have made counts of the number of mitochondria that occur in living amoebae, and there can be not doubt that in this organism the number varies greatly. For example, in seven amoebae the number present were: 17 (fairly large individuals), 28 (fairly large), 14 (fairly small), 15 (small individual), 30 (fairly large), 12 (small), 17 (fairly large). In this series there was certainly no indication of constancy of number of mitochondria, and one large individual even contained fewer mitochondria than another that was larger than itself.

Similarly, I find that in opalina, where the mitochondria are present in very large numbers, no indication of a constant number can be observed.

SUMMARY.

1. A dye-stuff (sodium salt of diethyl safranine monocarboxylic acid), formed from janus green by hydrolysis of the nitrile, is found to be selectively absorbed by mitochondria. Several bacteria have been tested with this substance, but they do not show a corresponding selective absorption.

2. Mitochondria of an heterotrichan infusorian, stained by this means, have been observed to undergo binary fission. The process occupies about four minutes. Fission of the unstained mitochondria is also described in amoeba.

3. In amoeba fusion of mitochondria has been observed.

4. Observations have been made in living amoeba on the relation of mitochondria to the process of intracellular digestion. The engulfed food circulates in the protoplasm, and mitochondria come into direct contact with it; they adhere to the food, and finally a vacuole is secreted around the food, with its adhering mitochondria. The mitochondria slowly dissolve, and the food undergoes disintegration. The vacuole slowly decreases in size, thus indicating an absorption of the digested food. It is concluded that the mitochondria, which are believed to be of an enzymatic nature, have brought about the digestion of the food.

5. Neither in amoeba nor in opalina does a constant number of mitochondria occur in the organism.

I wish to acknowledge my indebtedness to Dr. O. W. Tiegs, of Melbourne University, for his suggestions during the course of this investigation.

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FURTHER REMARKS ON THE STRUCTURE OF THE SPINAL CORD

by

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The conception of successive neurones of a reflex arc, terminating the one upon the next, by branching, discontinuous synapses, which embrace the body of the nerve cell, or ramify among its dendrites, has become so generally accepted, mainly through the histological work of Ramon y Cajal, that the new interpretation which I have placed upon the structure of the neurone junctions (2, 3) may deserve further discussion.

The conclusion previously reached, was that the union of the neurones took place through the cell dendrites; this does not, of course, imply a dendrite anastomosis of the kind described many years ago by Gerlach, but on the contrary considers that the fine collaterals that pass from the white matter into the grey matter, enter the dendrites of the nerve cells either singly or in bundles, so that the integration will occur within the body of the nerve cell, and not on a synaptic membrane which is closely applied to the nerve cell.

The only convincing illustration of a synapse within the spinal cord is the well-known figure of Cajal (see Cajal's book, vol. I, figure 110), of three nerve cells of Clark's column, having each a large, branching, terminal arborization from a collateral spread over its surface. The preparation was made by the defective methylene blue process, a method which, as I have previously pointed out, yields some very confusing artefacts. The only other evidence for the existence of synapses within the spinal cord consists of certain illustrations of small conical "knobs," closely applied to the body of the nerve cells. But little weight can be attached to this observation, since the "knobs" have never been shown to be connected with nerve fibres.

The whole question of the structure of the neurone junctions of the cord may best be understood by reference to the accompanying figure of a transverse section through an entire half of the spinal cord. The figure has been drawn from a section of the cord 25μ in thickness, and stained by the reduced silver method of Cajal, using alcohol as the fixative, and hydroquinone as the reducer.

The preparation was an extremely successful one, the whole of the nerve cells, with their axons and dendrites being completely stained. The completeness with which the staining has taken place is best appreciated by observing the white matter, in which an enormous number of closely packed longitudinally running nerve fibres have been blackened by the stain. These fibres, as the figure shows, are of varying thickness, and fibres of the same thickness usually tend to run together. Furthermore, if we follow any of the axons or "dendrites" from the various nerve cells in the grey matter, we can often trace them over very great distances, and when such axons or "dendrites" end, their ending is really a false one, for they have merely been cut short as they emerge from the plane of the section, and no true axon or dendrite termination can be observed. This fact is very readily made out by focussing the microscope up or down at the point where the fibres end, when such ending will invariably be found to be accompanied by the fibres passing out of the section. Since no natural termination of axons or "dendrites" can be demonstrated, we are justified in concluding that they have become very completely stained. Finally, it is possible to conclude that practically every nerve cell has been stained. Incompletely stained preparations always show numerous unstained nerve cells, obviously recognizable through the appearance of their nuclei, such nuclei being very clearly distinguishable even when they are quite unstained. In the preparations from which the accompanying illustration was drawn, only an occasional unstained nerve cell occurred. Finally, although the nuclei of the neuroglia cells have stained (they are not included in the figure) the neuroglia fibres have not been rendered visible, so that no opportunity has arisen for confusing fibres of neuroglia cells with nerve fibres. From these considerations, then, we are justified in concluding that the entire system of nerve fibres, or nearly the entire system, within the section, has been rendered clearly visible by the stain.

It is customary to represent "synapses" in two different ways: either as branching endings of nerves that ramify among the dendrites of a nerve cell, or as branching endings that spread over the "body" of the nerve cell, and embrace it closely, but do not come into direct contact with it.

The former mode of representation of a synapse is found in most diagrams illustrating the structure of the reflex arc of the spinal cord, and Cajal himself gives schematic figures of synapses conceived in this manner (see Cajal's book, vol. II, figure 549). The impossibility of this view is best illustrated by reference to the accompanying figure of the section through the spinal cord. In many of the nerve cells, especially those of the anterior horn, the "dendrites" can be followed for very long distances away from the "body" of the nerve cell, and some of the nerve cells spread over half the area of the section. The synapse, conceived as a branching nerve termination, ramifying among the "dendrites" of the nerve cell, would have to be of similar dimensions. As no histologist has

ever claimed to observe such a structure, it is safe to say that this mode of representing synapses is entirely unjustified.

As evidence for the second mode of representing a synapse, we have, as far as I am aware, a single figure, that drawn by Cajal from a methylene blue preparation (see Cajal's book, vol. I, figure 110). In this well-known figure several collaterals are shown, terminating by branching arborizations on nerve cells from Clark's column. But a possible alternative explanation for this figure is, as I have pointed out before (3), that methylene blue often yields serious coagulation artefacts, and such artefacts, when represented on paper, may be indistinguishable from branching synapses. On the other hand, the section through the spinal cord here given, leads to an entirely different conclusion. The drawing is from a reduced silver preparation, in which, as discussed above, practically the whole of the neurones seem to have stained; a denser mass of fibres than that shown in the figure could scarcely be expected. Nevertheless, on not one of the many nerve cells can a single synapse be observed. The experimental work of Sherrington has shown that when a neurone ends upon another neurone of a reflex arc, numerous other neurones must also converge upon this second neurone, so that this second neurone will be the servant of not one, but of many neurones afferent to it (Sherrington's Principle of Convergence). If Cajal's interpretation of a synapse were correct, we should be justified in expecting the preparation, the *whole of whose nerve elements have stained*, to show a dense cluster of branching synapses embracing the bodies of all the nerve cells. But not the slightest indication of such a cluster can be detected. On the contrary, the fine fibres which pervade the grey matter enter the dendrites of the nerve cells singly or in small bundles, and having entered the cells, become continuous with, and are indeed indistinguishable from, the neurofibrils of the nerve cell. This has been clearly illustrated in my two previous papers, and need not be further discussed here.

In attempting to verify these observations on preparations of the spinal cord; instances will often be observed of nerve fibres that end fairly close to the nerve cells. But careful examination will invariably show that they are only fibres which have been cut short as they left the plane of section of the preparation.

A more serious objection might be raised against the conclusions here reached, namely, that although it is often possible to trace the fine fibrils outwards from the nerve cell, sometimes for very long distances, yet it is seldom possible to trace them into the white matter; are we therefore justified in regarding them as neurones that have grown into the nerve cells, or must we consider them, as Cajal did, merely as enormously long dendrites that have grown out from the nerve cells, and extend over long distances in the cord? It is in the failure to recognize this distinction that the whole misinterpretation of neurone junction structure in the cord has arisen. Firstly it is to be noticed that when we trace the fibrils outwards from the nerve cells they are never observed to have any

actual termination, but their ending is always due to their leaving the plane of the section; we should have to conclude that the "dendrites" were of indefinite length. Secondly, it often happens that we can trace one of the fibrils into the white matter, but it is only rarely that we can do this for more than one fibril; but when we observe the irregular path that the fibrils take as they travel through the grey matter it is obvious that it is only an occasional fibril that we might reasonably hope to trace for any considerable distance. Still fewer nerve cells would be found in which we could trace more than one fibril right into the white matter. In such sections of the spinal cord, indeed, we can seldom trace even the axons for any great distance; nor is it usually possible to trace any individual collateral far from the white matter into the grey, and, as we might expect, we also find in the grey matter numerous fibres which cannot be shown to have any relation whatever to nerve cells; but in all such cases, examination under high magnification shows that the fibres have been cut short during the preparation of the sections, and that their endings are only artificial. Thirdly, if the fine fibrils were merely cell dendrites that have grown out from the nerve cells, we might expect them to have the appearance of protoplasmic outgrowths from the nerve cells, the cell membrane that covers the nerve cell being continued over them just as this membrane covers the axon that grows out of the cell. But if well-stained nerve cells are examined it will be observed that such a cell membrane is absent; the preparations, indeed, seem to show that these fibrils have either grown into the nerve cell from without, or that they have been formed by outgrowth from the neurofibrils of the nerve cell, but not that they are enormously elongated outgrowths from the walls of the body of the nerve cell. This fact is made very clear by employing the reduced silver process, but it is impossible to reveal it by the cytologically crude Golgi method, and it is on this latter method that Cajal has in the main relied for his elucidation of the structure of neurone junctions.

From these considerations we must conclude that, while it is not usually possible to trace the fibrils that appear to grow into the nerve cells, outwards from the white matter, from which they enter the grey matter, yet it is equally impossible usually to trace individual undoubted collaterals very far into the grey matter, since their path so seldom approaches that of a straight line. Nevertheless, these collaterals must have some relation to the nerve cells of the grey matter; and when we observe fine fibrils (though they cannot usually be traced individually into the white matter) approaching the nerve cells as components of bundles of fibres (many of which are undoubtedly neurone collaterals) and when we further observe that these fibrils that now enter the dendrites of the nerve cells are identical in appearance with undoubted neurone collaterals, or branches of these neurone collaterals; and, lastly, when we find that branching synapses applied to the body of the nerve cells do not occur, we seem justified in concluding that the fine fibrils that enter the nerve cells by way of its dendrites

are identical with neurone collaterals. For example; in the accompanying figure the nerve cell indicated by "A" receives numerous fine fibrils by two of its dendrites from bundles of fine neurone collaterals that enter the cord through the substance of Rolando. We cannot trace these fibrils that have entered the dendrites outwards right into the white matter, for they leave the plane of the section, nevertheless, we seem justified in concluding that they are branches of the neurone collaterals that penetrate the substance of Rolando at B, for they are members of a bundle of fibres, many of which undoubtedly originate from the collaterals, and in appearance, moreover, are quite indistinguishable from these. The nerve cell at C receives fibrils from this same bundle, but it would be even more unreasonable to expect to trace these fine fibrils right into the white matter.

On Nerve Dendrites.

In reduced silver preparations the dendrites of the nerve cells are seen as thick outgrowths from the body of the nerve cells. These dendrites are of varying thickness and length, being sometimes short, at other times as long as the body of the nerve cell itself, and may even at times undergo a limited amount of branching. Into these dendrites the fine nerve fibrils pass, and usually no difficulty is experienced in distinguishing between dendrites and the fibrils that have grown into them. But in Golgi preparations the whole nerve cell, with its dendrites and fibrils, appears uniformly black, and we get the false appearance of nerve cells devoid of internal differentiation, and provided with enormous dendrites, that may sometimes extend nearly over the whole section of the cord. Even in reduced silver preparations, if these are too strongly stained, a somewhat similar effect may be obtained, and, indeed, in some of the anterior horn cells shown in the figure, apparently undifferentiated "dendrites" may be observed.

If we observe the general disposition of the true dendrites of the nerve cell we notice that they are not disposed irregularly and at haphazard within the grey matter, but on the contrary are in the direction of the small bundles of collaterals that traverse the grey matter, giving the appearance of processes that have grown out from the nerve cells in order to "collect" fibrils from various regions of the grey matter into the body of the nerve cell. In the nerve cell indicated by A this tendency is obvious; but usually the paths of fibrils are so irregular within the grey matter that the tendency to arrange themselves along the fibril paths becomes much obscured. This arrangement is especially difficult to represent in the drawing, since so much of it necessarily occurs in planes angular to the plane of the drawing. It is usually best observed at the edge of the grey matter, where well-defined fibril paths exist. Such a case is shown in figure 3, taken from a posterior horn cell, closely adjacent to the substance of Rolando. Three bundles of collaterals are seen entering the grey matter, and

the dendrites are disposed in the direction of these fibrils; some of the fibrils enter the nerve cell, but most of them pass over the nerve cell, and end short, being cut across as they left the plane of the section.

The difficulty of observing the relation between the collaterals and the nerve cells to which they are related is increased by the fact that the collaterals, shortly before entering the grey matter, may undergo considerable branching. This, at any rate, is the case in the dorsal fibres in the cord. The fibres that enter the grey matter from the white matter usually do so in massive bundles, so that it is not usually possible to observe to what extent branching of these fibres has occurred. Nevertheless, occasional fibres, if not obscured by others, seem to show branching very clearly; see, for example at D in the figure. This branching collateral is shown at higher magnification in figure 2. (Incidentally it may be noticed that this branching fibre has the appearance of a synapse. The resemblance is a superficial one, for the termination of the fibres is a false one, due to the fact that its fine branches are cut through in making the section, while in any case it "terminates" in the substance of Rolando, in which nerve cells do not occur.) The extensive branching undergone by the dorsal collaterals as they pass through the substance of Rolando seems to be indicated by the thick mass of very fine and irregular fibres that lie at the border of this substance and the adjacent portion of grey matter. A similar branching of dorsal collaterals is shown in many of the figures of spinal cord sections given by Cajal from preparations made by the method of Golgi.

SUMMARY.

A drawing is given of a complete transverse section through half the spinal cord, prepared by the reduced silver method of Cajal. In this preparation, in which, it is believed, the *whole* of the neurones are completely stained, no trace of branching synapses applied to nerve cells, or ramifying among their dendrites, can be detected. On the contrary, the collaterals, probably after undergoing branching, penetrate the dendrites of the nerve cell, and become continuous with its neurofibrils in the manner described in two previous papers (2, 3).

The dendrites of the nerve cells are so disposed as to enable them to "collect" into the nerve cell individual fibres from the bundles of collaterals that traverse the grey matter.

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Figure 2. A posterior collateral, undergoing branching. This is the collateral shown at D in the previous figure.

Figure 3. A nerve cell from the posterior horn, with dendrites disposed in the direction of three bundles of collaterals, some fibres of which penetrate the dendrites, though most of them pass over them.

Rabbit. Cajal reduced silver method.

VARIATIONS IN THE UREASE CONTENT OF DIFFERENT VARIETIES OF SOYA BEAN *(Glycine hispida)*

by

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(Submitted for publication 30th June, 1926.)

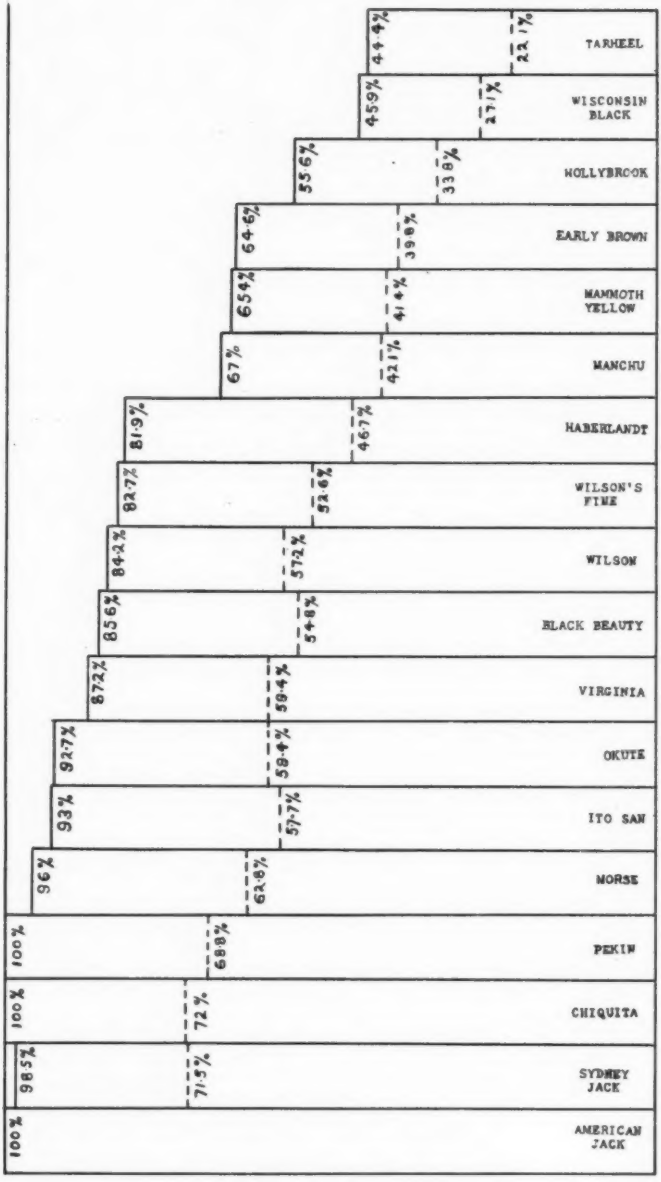
Since Takeuchi discovered the presence of urease in the soya bean this bean has been employed extensively for the detection and estimation of urea, particularly in urine and in blood. The urease converts the urea into ammonium carbonate quantitatively, and the ammonia can then be estimated by suitable means.

Annett (1914), working in Bengal, India, tested several varieties of soya beans, in order to see if there were any large variations in their urease activity. He distinguished these beans by the colourings of the seed coats, namely, soya bean yellow, soya bean spotted, soya bean chocolate, soya bean common, soya bean black, and Rymbsa Ktang, and he found that five of these six varieties were practically identical in urease activity; soya bean spotted was found to be distinctly more active.

Mateer and Marshall (1916) determined the relative amounts of urease in a number of different beans, and showed that the Jack bean *(Canavalia ensiformis)* contained about fifteen times as much urease as their soya bean. They also found that the sword bean *(Canavalia gladiata)* was much more active than the soya bean, but not as active as the Jack bean.

Other seeds and plant parts have since been examined, but there does not seem to have been any further investigation as to the relative urease activity of different horticultural varieties of the same bean.

For the determination of urea in this laboratory a Jack bean meal from America has been used, but since one lot of this deteriorated, and stocks were low, it was necessary to seek some other source of urease. The locally grown soya bean was obtained, and it was found that many varieties of soya bean are grown, differing in size and shape of the seeds and in the colour of the seed coat. Owjng to the kindness of the manager of the Glen Innes Experimental Farm, Mr. C. S. Sparks, and of Mr. Carne, samples of the seeds of sixteen varieties were obtained and examined. These may be classified as black, brown, yellow, and



Graph representing the % urea decomposed by the bean extracts in a 1% urea solution at 51°C.

——— represents % urea hydrolyzed in 60 minutes.
– – – represents % urea hydrolyzed in 30 minutes.

yellowish green, from the colour of their seed coats. Table 1 gives the names of the varieties and their colours.

TABLE 1.

Black.	*Brown.*	*Yellow.*
Pekin	Virginia	Chiquita
Wilson	Early Brown	Mammoth Yellow
Wilson's Fine		Manchu
Black Beauty	*Yellowish Green.*	Ito San
Wisconsin Black	Morse	Hollybrook
Tarheel	Okuter	Haberlandt

These beans were all grown on the same field, on the same kind of soil, at the Glen Innes Experimental Farm, and were all gathered shortly before they were examined, so the differences observed cannot be due to differences in the conditions of growth nor in the freshness of the seed.

To determine the urease activity of the beans the effect of extracts on a 1% solution of urea was observed.

To make the extracts 10 grms. of the finely-ground beans were added to 100 cc. of 30% alcohol, the mixture shaken from time to time, allowed to extract over night, and then filtered. Faintly opalescent extracts were obtained. In making the extracts 30% alcohol was used instead of water, since the solutions filtered so much more readily. Further, Marshall (1914) has shown that 30% alcohol does not appreciably retard the reaction between the urease and urea, nor is there much apparent destruction of the enzyme by alcohol of this strength.

To carry out the estimations 10 cc. of the filtered extract were added to 100 cc. of a 1% solution of urea, previously brought to the temperature at which the experiment was being conducted. The mixture was kept in small stoppered bottles, in order to reduce the likelihood of loss of ammonia. At intervals 5 cc. were removed as quickly as possible, and run into about 100 cc. of cold water. This was then immediately titrated with N/10 sulphuric acid, with methyl orange as indicator.

Experiments were carried out at 51°C. and 15°C., the bottles being immersed in a water-bath with a constant temperature regulator. Duplicate estimations gave good agreement.

TABLE 2.

	15 mins.	30 mins.	45 mins.	60 mins.
American Jack ..	94·0	100	100	100
Sydney Jack	43·2	71·5	88·0	98·5
Chiquita	42·7	72·0	93·6	100
Pekin	36·8	68·8	92·8	100
Morse	37·2	62·8	84·6	96

	15 mins.	30 mins.	45 mins.	60 mins.
Ito San	32·4	57·7	76·5	93
Okute	34·6	59·4	75·2	92·7
Virginia	35·0	59·4	75·2	87·2
Black Beauty	31·6	54·8	74·1	85·6
Wilson	37·2	57·2	72·2	84·2
Wilson's Fine ..	31·6	52·6	70·3	82·7
Haberlandt	29·3	46·7	69·5	81·9
Manchu	25·6	42·1	53·4	67·0
Mammoth Yellow ..	24·8	41·4	54·1	65·4
Early Brown	24·1	39·8	52·6	64·6
Hollybrook	18·8	33·8	44·4	55·6
Wisconsin Black ..	16·5	27·1	37·6	45·9
Tarheel	15·0	22·1	36·8	44·4

Percentage of urea decomposed by bean extracts in a 1% urea solution at times stated at 51°C.

Tables 2 and 3 give the percentages of urea decomposed at the times stated, when the temperatures were 51°C. and 15°C. respectively.

It will be seen from Table 2 that at 51°C. there is considerable variation in the urease activity of the extracts of different varieties of soya bean. In a period of thirty minutes the amount of urea decomposed varied from 22·1% to 72%. In an hour extracts of two of the varieties only had hydrolyzed the whole of the urea present in the solution, while the extracts of the other varieties hydrolyzed from 44·6% to 96% of the urea.

For comparison some of the Jack bean meal received from America, which had been in stock for some twelve months, and some Jack beans recently grown in Sydney were examined in the same way.

The American Jack bean was found to be very much more active than any of the soya beans, while the locally grown Jack bean was no better than the best of the soya beans.

To what the differences in the urease content of the different varieties of soya bean are due is not clear, but the fact that such great differences do occur is of interest, and makes it clear that before using a sample of soya bean or of Jack bean for the estimation of urea its activity should be determined.

TABLE 3.

	2 hours.	3 hours.	4 hours.	5 hours.	24 hours.
American Jack ..	59·8	69·0	83·0	91·0	100
Sydney Jack ..	41·0	54·0	62·0	71·0	100
Chiquita	36·6	45·5	54·5	63·4	100

	2 hours.	3 hours.	4 hours.	5 hours.	24 hours.
Morse	33·2	43·3	52·0	60·4	100
Pekin	30·6	40·3	47·8	56·0	100
Wilson	25·7	34·3	42·6	50·0	100
Wilson's Fine ..	25·4	34·3	41·0	50·0	100
Wisconsin Black ..	23·5	31·3	35·5	45·7	77
Tarheel	21·0	23·9	27·6	32·5	70·5

Percentage of urea decomposed by bean extracts in a 1% urea solution at times stated at 15°C.

Table 3 shows the activity of a number of the bean extracts at room temperature (15°), and these experiments, like those at 51°C., show that there are considerable differences in the urease content of the various beans.

The amount of urease hydrolyzed varied for the different soya beans from 32% to 71·1% for a period of five hours.

At room temperature (15°C.) the activity of the urease is markedly lower than at 51°C., and at the end of five hours, even the much more active Jack bean had not decomposed the whole of the urea present in the solution, whereas at 51°C. it had converted the whole of the urea into ammonium carbonate within half an hour.

SUMMARY.

The urease activity of eighteen varieties of soya bean was determined, and considerable variation was obtained.

At 51°C. 1 grm. of urea was completely hydrolyzed by 10 cc. of 10% bean extract within half an hour by two varieties of soya bean, namely, Chiquita and Pekin, while only 44·4% of the urea was acted upon by the variety Tarheel; the other varieties of soya bean tested had a urease activity intermediate between these.

The urease activity of the beans examined showed no relation to the general appearance of the seeds; the size and shape of the seeds varied, as also did the colour of the seed coats.

An American Jack bean was tested, and found to be much more active than any of the varieties of soya bean, but a Jack bean grown locally in Sydney showed practically the same urease content as the more active varieties of soya bean.

The reaction at room temperature was not complete within five hours, although the Jack bean had completely hydrolyzed the urea present in the solution within thirty minutes at 51°C.

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THE ELECTROLYTIC PREPARATION OF CALOMEL FOR USE IN THE CALOMEL ELECTRODE

by

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The calomel electrode is universally used as a standard half-cell in conjunction with the hydrogen electrode for precise determinations of P_H, oxidation-reduction potentials, and such. For a strictly reproducable standard half-cell, finely divided and very pure calomel is essential. In the method of preparation here outlined the calomel is made in the presence of N/10, N, or saturated KCl, as desired, the product is fine-grained and intimately mixed with mercury, and the process is less tedious and more easily handled than the usual electrolytic processes employed. In the usual methods of preparation (see, for example Hulett and Lipscomb (3) and Ewing (2)), the calomel is precipitated on a mercury surface, and continually wiped away with some stirring device. In the present method a mercury dropping electrode forms the anode, and the calomel is precipitated on the falling mercury stream, which carries down a fine layer of calomel, and at the same time continually presents a new mercury surface to the current. The arrangement can be followed from figure 1.

Into the vessel (A) is poured some pure mercury, and then a solution of KCl (normal, N/10, or saturated, according to the electrode required). B is a 400 cc. beaker, containing a saturated solution of cupric chloride, into which dips a copper electrode to form the cathode. A and B are connected by means of the bridge (C), which has a minimum diameter of 2 cm., this minimum being necessary to prevent any heating in the bridge. The lower extremities of the bridge are filled to a depth of 2 cm. with agar-gel, 2% agar in saturated KCl. The rest of the bridge is filled with a saturated solution of KCl. Controls showed that no copper nor hydroxyl ions diffused through the bridge during the process. The mercury dropping from the funnel (D) forms the anode, and has a platinum wire dipping into it, connected to the lighting circuit through an ammeter, and a suitable resistance.

Various methods of dropping were tried, in which the size and rate of the drops were varied. The most satisfactory results were obtained by allowing the

mercury to flow through a piece of silk mesh (E) tied to the end of the funnel, whence it emerged as a very fine spray. As soon as the current is thrown on

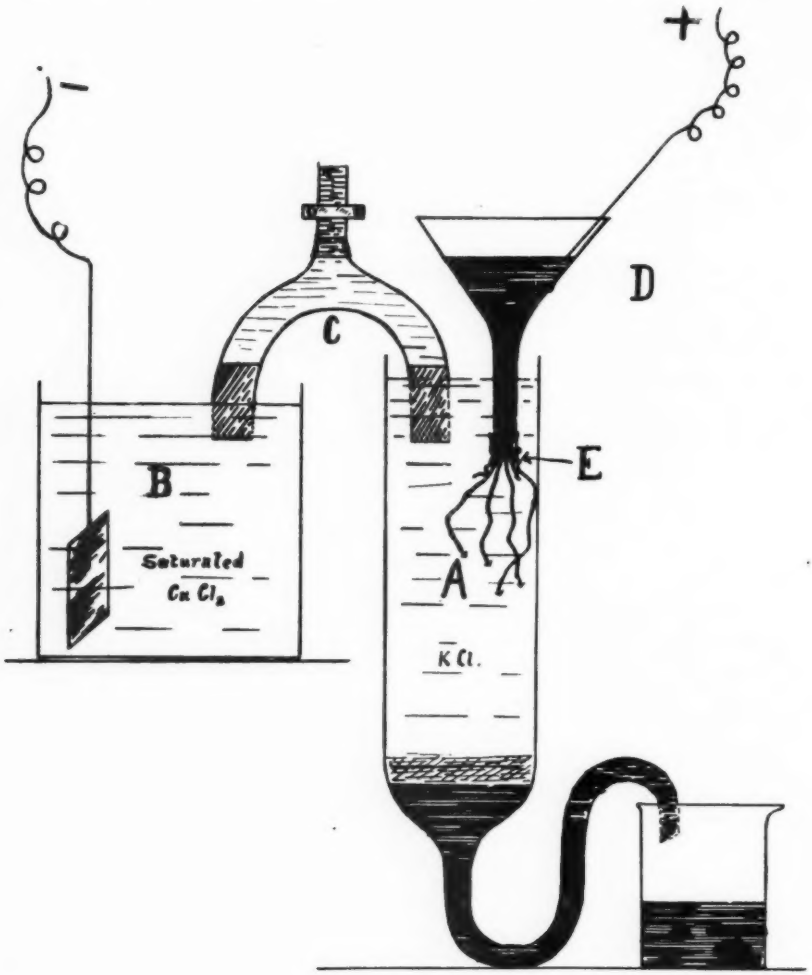

Figure 1.

the innumerable tiny drops coalesce into a dozen or so threads, as shown in figure 2. This effect is very striking and convenient, for these ever-changing threads continually present new surfaces to the solution whilst connected to the

source of mercury supply and to the current. These threads have a maximum length, if the current is small, i.e., less than $\frac{1}{4}$ ampere, but in such cases the collected calomel is so intimately mixed with a relatively large bulk of mercury that it is difficult to get a calomel layer. It is preferable to use larger currents (1 to 2 amperes) when the emerging threads are much shorter, but the yield is much larger and more readily separated from the bulk of the mercury.

In this work the action was started with a small head of mercury in the funnel and a current of about $1\frac{1}{2}$ amperes. As the action proceeded the mercury flow gradually fell off, due to a partial clogging of the silk, and then became fairly steady. At this stage the current was cut down to $\frac{3}{4}$ of an ampere, and the funnel filled with mercury, whence little further attention was necessary. A fine white sample of calomel, overlying a grey mass of finely divided mercury and calomel, was obtained, and two runs each of forty minutes yielded sufficient calomel for a dozen ordinary calomel electrodes. Eight such electrodes were made up, and gave strictly reproducible and correct values. Four of them were used in the chain below:

$$H_2, Pt,\ 0{\cdot}1N\text{–}HCl,\ \text{sat.}KCl,\ N\text{–}KCl, Hg_2Cl_2,\ Hg,$$

and they gave E.M.F. values of ·3483, ·3484, ·3483, and ·3483 volts.

During the above method of preparation the KCl in the anode chamber varies in strength. The collected calomel must therefore be well washed, and allowed to stand in contact with the standard solution of KCl of the required strength.

One advantage of this method is that we initially require pure samples of only two substances, KCl and mercury. The former can be purchased in the very pure state, but is generally recrystallized twice before use. The purest mercury can be recovered even from the most contaminated of laboratory stock in the following manner. The impure mercury is first run through a fine hole in a filter paper, and the gross impurities rejected. The sample is then well shaken with several lots of distilled water, and after the water is poured off the mercury is heated to boiling point in a beaker. It is then poured through a small funnel with a very long stem, the stem having been turned up for about 4 inches of its length and then turned down about the same distance, to end in a narrow orifice. The efficiency of this funnel in removing adhering contaminations is quite remarkable, and if the sample to be purified contains only such contaminations, and has not been used with substances that react with, or dissolve in, or form amalgams with, the mercury, then the process of purification can stop at this point. Various tests carried out in this laboratory have shown that in such cases this funnel delivers mercury which has all the properties of the pure distilled samples. If, however, the sample contains dissolved impurities, these are removed by spraying the mercury through a long column of 10% KOH solution, and then through a similar column of HNO_3 of density 1·1. Usually

the mercury is sprayed from the fine point of a funnel, but a piece of silk tied over the end of a wide-stemmed funnel gives about a hundred times the efficiency. The mercury should be passed through these solutions at least twenty times. At this stage the mercury could be heated and dried, and then passed through the bent-stemmed funnel again, whence the product could be generally classified as very satisfactory, and suitable for all ordinary purposes. When the highest

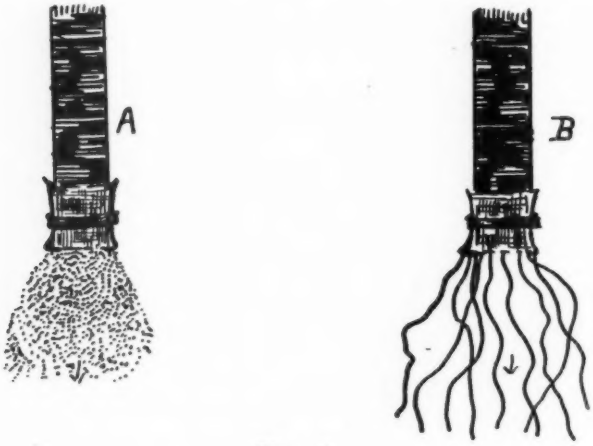

Figure 2.

degree of purity is required, as, for example, in the preparation of the standard calomel cell, it is necessary to distil the purified sample, generally in vacuum. Of the various stills in use, the simplest to construct and to maintain is the self-pumping type described in Clark (1). Although it distills slowly, it has the advantage of requiring practically no attention. Such a still in this laboratory produces about 3 lb. of mercury per day, and the only attention it receives is the switching on of the current to the heating element in the morning and the switching off again at night.

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THE ACTION OF CERTAIN FAT-SOLVENTS ON ALCOHOLIC FERMENTATION

by

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(Submitted for publication 18th May, 1926.)

The action of toluene on fermentation by yeast cells has been studied by Harden (2, 3), who found that the addition of toluene to a fermenting mixture of yeast cells and sugar causes a rapid fall in the rate of fermentation, until a relatively constant rate is attained much lower than that of the original mixture.

He further showed that the yeast acquires the property, not possessed by living yeast, of reacting to added phosphate in a manner similar to that of yeast-juice and dried yeast preparations. Thus in presence of toluene the addition of a soluble phosphate causes an increase in the rate of fermentation of sugar, which persists for a time, and then gradually falls practically to the original rate. During this period of enhanced fermentation the phosphate is converted into hexosephosphate, and Euler and Johansson (1) have used toluened yeast for the preparation of this substance.

Toluened yeast, however, reacts to phosphate less markedly than yeast-juice, but the property may be much increased by washing the yeast at the filter pump with toluene (3).

Another effect observed by Harden and Paine (4) is an increased autofermentation. The autofermentation of yeast is due to the fermentation of glucose produced from the glycogen of the cells by a diastatic enzyme, and as no increase in autofermentation results from the addition of toluene to yeast-juice, the effect on yeast cells cannot be attributed to direct action of the toluene on this enzyme. The action is considered by Harden as being probably due to the disorganization of the cell by the toluene, whereby the diastase finds more ready access to the glycogen.

He suggests that a similar action may also account for the action on the sugar fermentation, which may be associated with some special portion of the cell. Disorganization of the cell may produce altered conditions less favourable to fermentation, such as dilution.

On this hypothesis it may be assumed that when phosphate is added to yeast,

although it is able to pass into the cells (6), it does not penetrate to that portion where fermentation occurs. In the cells altered by toluene it is able to come into contact with the fermenting complex, and so take part in the reaction as it does in the completely disorganized yeast-juice.

In the hope of throwing further light on the subject, experiments have been carried out on the action of toluene, and also of other substances having similar solvent properties, and like toluene being slightly soluble in water.

The samples of yeast employed were top yeasts, and were prepared by washing repeatedly in the centrifuge with water and pressing in a small hand-press.

The fermentations were carried out at 30°C., and the carbon-dioxide evolved measured at frequent intervals of time in the apparatus described by Harden, Thompson, and Young (5). The mixtures were previously saturated with carbon-dioxide at the temperature of the bath.

Effect on the Fermentation of Glucose.

In each case 10 grms. of pressed yeast were added to 20 cc. of water and 4 grms. of glucose, and 5 cc. of the liquid added. The following were tested: Toluene, benzene, xylene, ether, chloroform, ethyl acetate, amyl acetate, ethyl butyrate, butyl alcohol, and a sample of petroleum ether boiling below 60°C.

Of these an action similar to that of toluene was observed with benzene, xylene, ether, and ethyl butyrate. In each case the rate of fermentation rapidly fell, until a constant rate was attained. Examples are given in Table 1, with toluene, benzene, and ether, the figures referring to cc. of carbon-dioxide evolved in successive periods of five minutes. The experiments were done with different samples of yeast; 10 grms. of yeast in the presence of sugar usually gave from 40 to 50 cc. in five minutes.

TABLE 1.

Toluene.	Benzene.	Ether.
22·0	12·8	22·5
15·8	7·3	4·6
5·5	5·7	3·5
4·2	4·8	3·0
2·9	4·5	3·3
2·0	4·7	3·5
2·0	4·5	3·3

Complete cessation of fermentation was brought about by the addition of chloroform, ethyl acetate, amyl acetate, or butyl alcohol.

Petroleum ether reduced the rate of sugar fermentation, but not to the same

extent as the other substances. In one experiment the addition of 5 cc. to 5 grms. of yeast in presence of sugar reduced the rate from 28 to 9·7 cc. in five minutes.

In another experiment yeast was stirred into about 100 cc. of petroleum ether, and after one hour filtered at the pump; 3 grms. of this gave a rate of 4·6 cc. in five minutes, as against 12·1 cc. for the untreated yeast.

Reaction to Phosphate.

For these experiments mixtures of 10 grms. of yeast, 20 cc. of 20% glucose, and 5 cc. of the liquid being tested, were incubated in the bath until the constant rate was attained; 5 cc. of a solution of sodium-phosphate (Na_2HPO_4 $12H_2O$) approximately 10%, and previously saturated in the bath with CO_2, were then added, and the rate observed every five minutes.

In most cases a typical reaction was obtained, the rate increasing to a maximum, and then rapidly falling to a constant rate, usually slightly higher than that of the original mixture. The additional carbon-dioxide produced during this period was obtained from the total fermentation and the final rate. The phosphate solutions employed were estimated by magnesium-citrate mixture, and the last column in the table gives CO_2 calculated from this (Na_2HPO_4 to CO_2).

The agreement was in general satisfactory.

A number of results are given in Table 2, the rates being given in cc. per five minutes.

TABLE 2.

Substance.	Initial Rate.	Maximum Rate.	Final Rate.	Extra CO_2 at N.T.P. Found.	Calctd.
Toluene, 1	3·3	9·2	4·8	40·4	43
„ 2	3·2	10·0	3·3	44·1	43
Benzene, 1	3·1	14·8	4·6	44·3	43
„ 2	2·8	11·1	3·0	44·1	43
Ether, 1	4·7	17·6	6·2	53·1	43
„ 2	2·6	14·0	3·9	49·0	43
Ethyl butyrate ..	3·0	12·5	3·4	40·1	43

Another series of experiments was carried out, in which the pressed yeast was stirred into about 200 cc. of the liquid, and allowed to stand for about fifteen minutes. It was then filtered at the pump and spread out on a sheet of filter paper, and allowed to stand for about an hour.

Weighed portions of this (usually 10 grms.) were fermented with 20 cc. of water, 4 grms. of glucose, and 5 cc. of phosphate added.

When treated with ether in this manner the yeast became very sticky, owing to plasmolysis, and in the later experiments 10 grms. of yeast were treated

directly in the fermentation flask with the ether. After fifteen minutes the ether was drained off, and a current of air blown through the flask to remove as much ether as possible. The solutions were then added to the flask, and fermentation commenced.

TABLE 3.

Substance.	Initial Rate.	Maximum Rate.	Final Rate.	Extra CO_2 at N.T.P. Found.	Calctd.
Toluene, 1	3·6	9·5	3·7	41·2	42·3
„ 2	3·0	7·0	3·9	44·1	43·0
Benzene, 1	3·3	16·8	5·5	40·4	43·0
„ 2	3·1	14·8	4·5	41·0	43·0
Xylene, 1	5·1	8·3	5·2	40·6	43·0
„ 2	2·5	9·4	4·8	44·9	43·0
Ether, 1	6·2	26·2	6·7	45·1	43·0
„ 2	7·0	22·5	7·2	44·6	43·0

With ether, either when added to the mixture or when the yeast was treated beforehand with the reagent, there was a marked decrease in rate on the addition of the phosphate, and the maximum rate was only attained after some time. This slow increase to the maximum introduces an error into the calculation of the extra carbon-dioxide given off in the phosphate reaction. In one instance yeast, after treatment with ether, gave a rate with glucose of 6·8 cc. per 5 minutes. On addition of 5 cc. of phosphate the rate fell to 2·6 cc., and then gradually rose to a maximum of 27·8 after sixty minutes. It then rapidly fell to a final constant rate of 6·8 cc. The agreement between the calculated and found values for the CO_2 were therefore not so good in the ether experiments. In no case was a reaction to phosphate obtained with petroleum ether.

Table 4 shows that prolonged treatment with toluene did not destroy the activity of the yeast. Ten grms. of the treated yeast were used in each experiment, and 5 cc. of phosphate were added.

TABLE 4.

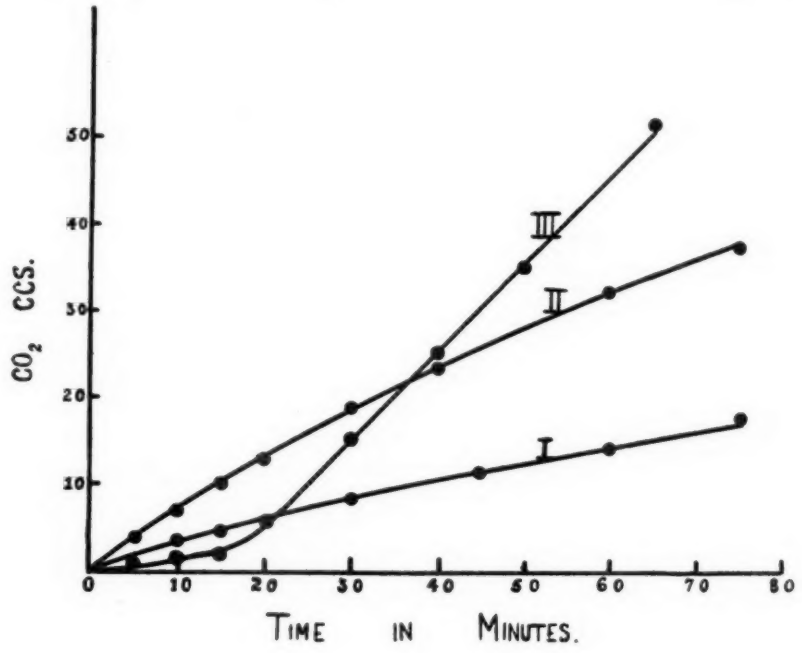
Time in Toluene.	Initial Rate.	Maximum Rate.	Final Rate.
15 minutes	3.6	9·5	3·7
30 minutes	3·8	10·8	3·9
15 hours	2·4	5·7	2·3

Effect on the Autofermentation.

The effect on the autofermentation was tried with petroleum ether, toluene, and ether, petroleum ether being tried because of its less marked action on the fermentation of sugar.

The Australian top yeasts employed showed a similar increase in the rate of autofermentation on the addition of toluene, to that described by Harden and Paine (4).

Ether exhibited the same effect, but in a more marked degree. In every case there was a period following the addition of ether, during which only a very feeble fermentation took place; then the rate increased, until a fairly constant rate was attained, which was higher than that given in presence of toluene.



These effects are seen in the accompanying curves, which refer to the following mixtures:

I. 10 grms. yeast + 25 cc. water.
II. 10 grms. yeast + 25 cc. water + 5 cc. toluene.
III. 10 grms. yeast + 25 cc. water + 5 cc. ether.

When petroleum ether was added to yeast and water the rate for the first few periods was usually high. This was probably due to the sample containing paraffins of low boiling point, which were vaporized at the temperature of the bath, especially when the flasks were shaken. After about thirty minutes, however, the rate became constant, and in the figures quoted the readings in the first hour have been discarded.

In one experiment the autofermentation of 10 grms. of yeast was 7·8 cc. in 40 minutes, whilst in the presence of 5 cc. of petroleum ether 43·6 cc. were obtained in the same time.

In another instance yeast was treated with petroleum ether for one hour, filtered at the pump, and the excess removed with a current of air; 5 grms. of this with 20 cc. of water gave a rate of 1·9 cc. per 5 minutes; 5 grms. untreated yeast gave 0·5 cc. per 5 minutes.

Effect on the Permeability of the Cell.

Yeast was washed with water in the centrifuge until the washings gave practically no reaction for phosphate. The yeast was then pressed. Two lots each of 10 grms. were treated with ether and toluene respectively by stirring with the liquids, standing for fifteen minutes, and then pouring off and draining.

To each was added 50 cc. of water, the mixtures shaken and allowed to stand for fifteen minutes, and centrifuged. The liquid was poured off, filtered, and the free phosphate estimated in 30 cc. of each, with magnesium-citrate mixture.

The following results were obtained:

TABLE 5.

	P_2O_5 in 50 cc. Filtrate.
10 grms. yeast untreated	0·0007 grms.
10 grms. yeast after toluene	0·0091 grms.
10 grms. yeast after ether	0·0381 grms.

In another series of experiments, lots of 10 grms. of yeast were treated as before with toluene and ether respectively. To each were added 20 cc. water, 4 grms. glucose, and 5 cc. of phosphate, the flasks were connected with the fermentation apparatus and incubated in the bath until the phosphate reaction had subsided. They were then centrifuged, and the free and total phosphate estimated in the supernatant liquids.

The figures obtained in three such experiments are given in the table, and represent P_2O_5 in 25 cc.:

TABLE 6.

	Free.	Total.
1. After toluene	0·0011	0·0752 grms.
After ether	0·0022	0·2165 grms.
2. After toluene	—	0·1385 grms.
After ether	—	0·1840 grms.
3. After toluene	0·0052	0·0894 grms.
After ether	0·0092	0·1488 grms.

These figures show that after treatment with either ether or toluene, both free and combined phosphates readily pass out of the yeast cells, but the permeability is more affected after ether than after toluene.

Further evidence that the yeast cell is more disorganized by ether than by toluene is furnished by the fact that after treatment with ether the coferment of alcoholic fermentation is readily washed out of the cell by water, whereas after toluene this does not happen.

Fifty grms. of pressed yeast were stirred up in a centrifuge vessel with 100 cc. ether, and allowed to stand, with occasional shaking, for fifteen minutes. The ether was then poured off, 100 cc. of water added, and shaken from time to time for about twenty minutes. It was then centrifuged, and the liquid decanted. The residue was further washed three times with water in the centrifuge.

Three lots of 10 grms. of the moist residue were weighed into flasks, and the fermentations measured in the presence of the following:

1. 2 grms. glucose + 25 cc. water.
2. 2 grms. glucose + 25 cc. first washings, after boiling.
3. 2 grms. glucose + 25 cc. water + 2·5 cc. phosphate.

The first gave no fermentation in twelve hours; the second showed an active fermentation, giving 77 cc. in the first hour.

The absence of fermentation in the third mixture showed that the loss of activity was not due to the washing out of phosphate.

In a second experiment, the yeast, after treatment with ether in a similar manner and subsequent washing, was pressed in the small hand-press. 10 grms. of this with water and sugar gave no fermentation in seventeen hours, whereas in the presence of the washings over 200 cc. were evolved in the same time.

Yeast treated with toluene and then repeatedly washed with water, was found to ferment glucose quite as actively in the presence of water as in that of the washings.

Treatment with ether, and subsequent washing with water, forms a very convenient method of preparing the alcoholic ferment free from coferment.

Preparation of the Hexosephosphates.

As the yeast cell after treatment with ether readily allows the hexosephosphates to pass out into the surrounding fluid, these substances can conveniently be prepared by this means. A weighed quantity of pressed yeast is put into a large flask, covered with ether, and well shaken. After about fifteen minutes the ether is drained off as much as possible, glucose solution is added, and the flask placed in a bath at 30°C., and phosphate solution added from time to time.

To control the addition of the phosphate, 10 grms. of the yeast are treated in an exactly similar manner, connected with the fermentation apparatus, and

a corresponding amount of phosphate added. The rate of fermentation of this indicates when the reaction due to each addition of phosphate is completed, and the quantity which may be added. When the reaction is over the mixture is centrifuged, and the clear liquid employed for the preparation of the hexose diphosphate, as described by Young (8), or for both the monophosphate and the diphosphate, as described by Robison (7).

SUMMARY AND DISCUSSION.

1. Benzene, xylene, ether, and ethyl butyrate were found to have the same action on the fermentation of glucose by yeast cells as that already found by Harden for toluene, viz., a marked reduction in the rate.

Petroleum ether (below 60°C.) had a less marked effect; whilst chloroform, ethyl and amyl acetates, and butyl alcohol completely stopped the fermentation.

2. Benzene, xylene, ether, and ethyl butyrate, either when added to the mixture or when the yeast was previously treated with these liquids, caused the yeast to acquire the property of reacting to phosphate, with formation of hexosephosphate. This action was not found with petroleum ether.

3. Both ether and petroleum ether have a similar action to toluene in causing an increased rate of autofermentation, the effect of ether being the most marked.

4. When yeast was treated with ether or toluene the cells became more permeable to free phosphate and to hexosephosphates, and these substances could readily be washed out of the cells with water.

5. After washing yeast with ether, the coferment of alcoholic fermentation could be washed out of the cells with water. The yeast became inactive to glucose, and the fermentative activity could be restored by adding the washings to the mixture. This did not take place when toluene was used.

6. A method is given for preparing the two hexosephosphates with yeast which has been treated with ether.

The results suggest that yeast cells after treatment with petroleum ether, toluene, and ether represent three degrees of internal disorganization of the cells.

Petroleum ether, the least soluble in water of the three, alters the cell to the least extent. It increases the rate of autofermentation, decreases that of the sugar fermentation, but does not alter the cell sufficiently to give a reaction to phosphate.

Toluene has a greater action, so that it increases the rate of autofermentation, decreases that of sugar fermentation, the latter to a more marked degree, and the yeast is now able to react to phosphate. In addition it alters the cell wall so that phosphate and hexosephosphate may be washed out with water.

With ether the disorganization is more profound, so that in addition to those changes produced by toluene, the cell wall becomes permeable to the coferment, and this may be readily washed out with water.

Further evidence for this was given by the facts that all the cells were not killed by the treatment with petroleum ether, and cultures made into wort readily grew. On the other hand, in no case was any growth obtained when small pieces of yeast which had been treated with either toluene or ether were put into wort.

During the course of this work one of us (N.G.K.) has held a Research Scholarship of the University of Melbourne.

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THE ACTION OF ETHER ON THE YEAST CELL
I. CARBOXYLASE

by

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In the previous communication (2) it was shown that living yeast which has been treated with ether is altered in such a manner that the coenzyme of alcoholic fermentation may be readily removed by washing with water. The residue is quite inactive to glucose, and requires the addition of the washings in order that fermentation of sugar shall take place. It behaves, therefore, exactly like zymin (acetoned yeast) and other dried preparations of yeast.

It was shown by Harden (1) that such preparations were able to transform pyruvic acid into carbon-dioxide and acetaldehyde, even after they had been rendered incapable of fermenting glucose by removal of the coenzyme with water. As this action of carboxylase on pyruvic acid is generally considered to be one of the reactions in the change from glucose to carbon-dioxide and alcohol, it is clear that the coenzyme is not concerned with this stage in the fermentation of glucose (see also Neuberg and Gottschalk, 3).

The experiments described below showed that yeast which had been treated with ether, and the coenzyme subsequently removed with water, behaved in a similar manner to washed zymin.

In each case 50 grms. of top yeast were shaken up with about 100 cc. of ether, and after standing for fifteen minutes the ether was drained off as much as possible. The yeast was then shaken with 100 cc. of water, allowed to stand for thirty minutes, and the mixture centrifuged. The residue was further washed with water four times, finally suspended in 100 cc. of water, and this mixture used for the fermentations.

The first washings were boiled to remove the ether, and employed as coenzyme solution.

One experiment was tried with 0·25 gr. of pyruvic acid added to 25 cc. of the yeast suspension, but the mixture was too acid, and only a very slight action was obtained. The method used by Harden (1) was therefore employed, in which the pyruvic acid was neutralized with alkali, and the fermentation carried out in the presence of boric acid to decompose any alkali carbonate formed.

The following results were obtained:

Experiment 1. Date, 30/6/26.

1. 20 cc. yeast suspension + 25 cc. 1% pyruvic acid + 2·5 cc. N.NaOH + 3 gr. boric acid gave 29·2 cc. CO_2 in 75 minutes.
2. 20 cc. yeast suspension + 25 cc. water + 2 gr. glucose gave no fermentation.
3. 20 cc. yeast suspension + 25 cc. coenzyme + 2 gr. glucose gave 32·5 cc. CO_2 in 75 minutes.

Experiment 2. Date 7/7/26.

1. 25 cc. yeast suspension + 25 cc. 1% pyruvic acid + 2·5 cc. N.NaOH gave 18 cc. CO_2 in 50 minutes.
2. 25 cc. yeast suspension + 25 cc. water + 2 gr. glucose gave 0·8 cc. CO_2 in 50 minutes.
3. 25 cc. yeast suspension + 25 cc. water coenzyme + 2 gr. glucose gave 28·2 cc. CO_2 in 50 minutes.

In both experiments the formation of acetaldehyde was shown by the smell, and by the production of a colour with Schiff's reagent.

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A CONVENIENT HYDROGEN ELECTRODE

by

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The electrode about to be described was evolved during the course of some work on which the writer was recently engaged. The simplicity of its construction and the ease with which it may be used, where the hydrogen atmosphere

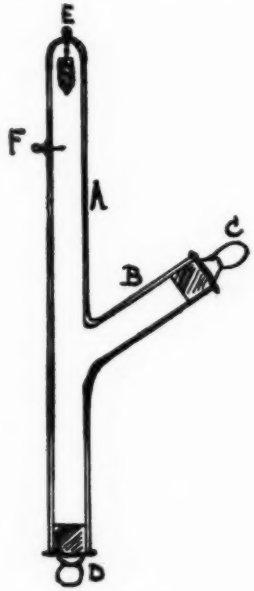

Figure 1.

employed is stationary, were such that it seemed worth while to enter into its description for the benefit of others to whom it might prove useful. As will be seen, the electrode tube may be regarded as a modification of the well-known form devised by Michaelis.

The Michaelis electrode depends for the completion of the chain between the hydrogen and calomel electrodes upon a tape or wick moistened with saturated potassium-chloride solution. Such wicks or tapes are often inconvenient to use, and it occurred to the writer that as it is possible to work with the tap of the Barendrecht calomel electrode closed, provided that it be not greased, it should be possible to design a hydrogen electrode which would complete the chain through the fluid present round an ungreased glass stopper.

As a matter of fact, there is sufficient capillary transport about the stopper in the Barendrecht calomel electrode when closed to permit the solution in the vessel to escape in slowly forming drops. This proves useful in preventing the diffusion of contaminating solutions back into the calomel electrode during use, and is thus of the greatest service.

The author's form of electrode consists of a vertical tube (A, figure 1), about 9 cm. in length and 1 cm. in diameter. To this, at about 3 cm. from its lower end, is joined a side tube (B), making an angle of about 45° with the main tube. Both the main and side tubes are closed by stoppers. The difficulty caused by having to grind in these stoppers may be avoided by cutting off the necks of suitably sized flasks, having stoppers already fitted to them, and fusing these to the electrode tubes.

At the upper end of the main tube (A) a platinum electrode (E), formed from heavy foil, is fused in position, and some little distance below this a piece of stout platinum wire (F) is also fused in. This latter is made to serve as the anode during the deposition of platinum black on the electrode proper. The exact form of the electrode (E) is not important, but it may be rolled slightly upon itself, and is shown in the illustration as having a pointed free end. This pointed end may be used to obtain "minimum contact" with the contained solution if so desired.

A convenient stand is shown in figure 2. In this the electrode tube is clipped by a spring in a V-shaped groove made in a block of brass, which in turn is freely adjustable as to height on a light steel rod. This standard is housed on a heavy foot, which is hollowed out as shown for the accommodation of a small beaker.

After the electrode is constructed it must be carefully cleaned by the usual methods to render it free from chemical impurities and grease, after which it will be ready for platinizing. The presence of the platinum wire (F, figure 1) renders the operation of platinization very simple, and the fixation of the electrode within the tube does away with the danger of contamination, which is always present when the platinum foil or wire has to be removed from the tube for this purpose.

The platinizing of the foil is effected by removing the stoppers from both main and side tubes, inverting the apparatus, and running in the platinizing solution until the wire (F) is completely immersed. The tube is now placed in its stand in the inverted position, E and F being connected to the negative and

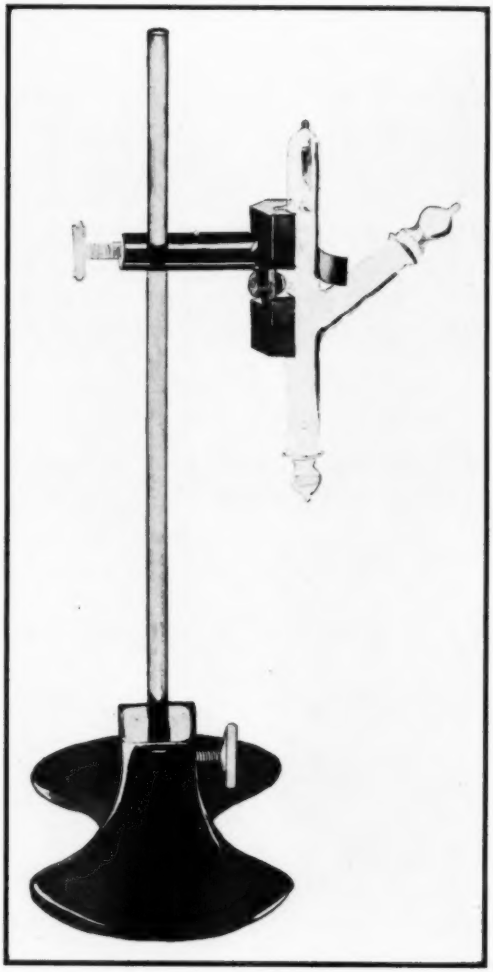

Figure 2.

positive terminals respectively of an accumulator, the coating and subsequent treatment of the electrode being carried out in the usual manner.

In measuring the hydrogen ion concentration of an unknown solution the stopper (C) is placed in position, and the solution is poured in at D until it is within a short distance of the opening. The stopper (D) is now inserted, and the tube is brought into the horizontal position with the side tube (B) uppermost. This allows the entrapped air to be caught in the tube (B). The electrode is now brought into the vertical position, with the electrode (E) uppermost, and the stopper (C) is removed. By means of a hollow platinum needle, such as is used in surgery, fused into a glass tube, hydrogen is bubbled into A. The gas displaces the liquid, which tends to fill the side tube (B). When the level of the fluid in A has fallen sufficiently for the end of the electrode (E) to be just covered, the platinum needle is withdrawn, and the side tube (B) is completely filled with the solution. Using a rotary motion, the stopper (C) must be so inserted that no bubble of air is trapped in the tube. The apparatus is now dried with a cloth, and is vigorously shaken with the hand for a few minutes, after which the stopper (C) is removed, and that at D is cautiously moved, so that it is completely wetted with the contained solution, and is kept in position by just enough pressure to prevent its dropping out. The tube is now clipped in position on its stand, and the carrier is lowered so that the stopper and end of the tube at D is well covered with a saturated solution of potassium-chloride contained in a small beaker. A fine wire from the negative terminal of the potentiometer is carefully hooked into the platinum loop at E, and on dipping the tube from the calomel electrode also into the small beaker below D, the chain is complete and a measurement may be made.

With this electrode a reading may be made which is sharp enough for most purposes, though, as would be expected, the results are more satisfactory when one is using the apparatus with solution of good electrical conductivity.